Soil dehydrogenase, acid phosphatase and urease enzyme activities in degraded and undegraded forest soils

6.1. INTRODUCTION

Biochemical reactions are the important nutrient transformation processes of organic and inorganic substances in soil environment through the catalytic activity of biomolecules called enzymes. Many of the organic matter transformation processes in soil are catalyzed by enzymes (Khan, 1970) and all biochemical transformations in soil are dependent on, or related to the presence of enzymes. The important sources of enzymes in soil include plant, animal and microorganisms. The quantity of soil enzymatic activity detected in a particular soil sample is the sum of active and potentially active enzymes (Tate III, 1995). However, the activity of a particular enzyme in the soil is a composite of various activities associated with various biotic and abiotic components, e.g. proliferating cells, latent cells, cell debris, clay materials, humic colloids and aqueous phase (Burns, 1982 and Tiwari et al., 1988a).

The measurement of biochemical activity in soil i.e. soil enzyme assays have been done for various reasons particularly, as a measure of soil fertility or productivity (Kiss et al., 1978; Dkhar and Mishra, 1983; Tiwari et al., 1988ab and Verstraete and Voets, 1977), as a measure of microbial biomass (Casida, 1977; Ladd, 1978 and Klose and Tabatabai, 1999), as indicators of vegetation effects of pollutants and capability to conduct bio-
geochemical cycling, total microbial activity (Stevenson, 1959 and Tiwari et al., 1988ab), as a predictor of bioremediation and potential success (Dick et al., 1998), to understand the consequence of rhizosphere effect (Boero and Thien, 1979), as a potential indicator of soil quality (Kennedy and Papendick, 1995; Garcia and Hernandez, 1997; Trasar-Cepeda et al., 1998; Bendick and Dick, 1999; Palma et al., 2000; Pascual et al., 2000 and Trasar-Cepeda et al., 2000). Research work on soil biochemistry during the last two decades appeared to be concentrated towards development of soil quality indices based on these biochemical properties. This is due to the reason that biological and biochemical properties are highly sensitive to environmental stress and thus can be used as indicators of soil quality (Trasar-Cepeda and Gill-Sotres, 1987; Dick and Gupta, 1994; Kennedy and Papendick, 1995 and Ajwa et al., 1999).

Microbial activities in soil, despite their importance in many of the soil processes, are frequently disturbed as shown by altered soil enzyme activities as a result of agricultural exploitations and tillage practices (Tiwari et al., 2002). Disruption in soil microbial activity as shown by changes in levels of metabolic enzymes, can serve as an estimate of ecosystem disruption (Tate, 1995). Among the different types of soil enzymes studied from various objectives of investigations, one oxidoreductase (dehydrogenase) and two hydrolases (phosphatase and urease) are thoroughly studied enzymes due to their specific importance in organic matter transformation processes, phosphorous cycle and agricultural practices. Soil dehydrogenase is an extracellular enzyme which is
considered to be a good tool to measure microbial oxidative activity (Ross, 1971), as an indicator of any disruption caused by pesticide application, trace element discharge and soil management practices (Versraete and Voets, 1977; Burns and Edwards, 1980 and Reddy and Faza, 1989) as a measure of microbial biomass (Ladd, 1978) and measure of soil respiration. Acid and alkaline phosphatase activity assays have been used to our understanding of the phosphorus cycling which is related to the organic matter and its turnover in soil (Speir and Ross, 1978 and Trasar-Cepeda and Gill Sotres, 1987). The abundance and activity of these enzymes in the soil is an indication of the available P as these enzymes are responsible for conversion of organic form of P to inorganic and labile P forms. Urease is another important and thoroughly studied soil enzyme due to the agricultural importance of its substrate, urea. The possible effects of soil properties and land use pattern on urease activity may have significant implications of the efficient use of fertilizers based on urea (O’Toole et al., 1982). However, little research has been done to determine the interaction between urease levels and environmental parameters in the soil (Stott and Hagedon, 1980). Reports have revealed that urease activity is directly related to type of vegetation and quality of incorporated organic materials and with fluctuations in nutrient levels due to associated changes in populations of urolytic microbes in the soil (McGarity and Myers, 1967 and Stott and Hagedon, 1980). Urease activity is an important factor for survival of ammonium fertilizer oxidizers in forest and agricultural soils (Swenson and Bakken, 1998).
The above research findings show that soil biochemical characteristics in terms of soil enzyme activities are largely affected by change in soil environment induced by disturbances such as deforestation, tillage, soil management and other agricultural practices. Therefore, this research investigation aims to study that if there exists significant variation in distribution of the soil enzyme activities (dehydrogenase, acid phosphatase and urease) in degraded, moderately degraded and undegraded forest soils of humid tropics in Arunachal Pradesh, north-eastern India.

6.2. Review of Literature

Detailed survey of the available literature on the studies of various aspects of soil enzyme activities reveals an extensive research work done in this field during the last three decades of the 20th century.

Research investigation on enzyme activity in gray wooded soil as affected by cropping systems and fertilizer use has shown that growing legumes in rotation resulted in considerably greater total microbiological activity than the wheat-fallow system (Khan, 1970). Further the study demonstrated that the enzymatic activity increased with increase in organic matter content in the soil.

Frankenberger and Bingham (1982) reported the inhibitory effect of increased soil salinity to the enzyme activities that have a specific role in the C, N, P and S cycles of saline soils. They also observed decrease in enzyme activity with increasing electrical conductivity or salinity, however, the degree of inhibition varied among the enzymes assayed and the nature and amounts of salts added. The activity of dehydrogenase was severely
inhibited by salinity, whereas, the hydrolases showed lesser degree of
inhibition.

An extensive study on the relationship between enzyme activities and
microbial growth and activity indices in different soils have revealed high
correlation of the enzyme activities with both microbial respiration and total
biomass in soil (Frankengberger and Dick, 1983).

Dormaar et al. (1984) studied the impacts of seasons and site
management on the enzyme activities of soils in Alberta, Canada. They
found highest enzymatic activity of soils in winter months and lower during
rainy season. Their results indicated the significant effects of grazing on
enzyme activities of soils in two sites.

Bolton et al. (1985) reported significantly higher levels of urease,
phosphatase and dehydrogenase following growth of winter peas (as green
manure crop) in comparison to the soils which received regular applications
of anhydrous ammonia, P and S at recommended rates for a period of 30
years in Palouse region of Eastern Washington.

Baruah and Mishra (1986) investigated the effect of three herbicide
(2,4-D, butachlor and oxyfluorfen) on activities of dehydrogenase, urease
and carbon dioxide evolution in submerged paddy fields of north-east India.
They noted significant stimulation of the dehydrogenase activity and carbon
dioxide output with herbicide treatments. However, they found that the
herbicide application on urease activity remained unchanged.

Study on enzyme activity and carbon dioxide evolution from upland
and wetland rice soils under three agricultural practices in hilly region of
north-eastern India revealed higher activity of dehydrogenase, urease, and carbon dioxide evolution in wetland (Valley soils), followed by terrace system and hill-slope site respectively (Tiwari, M.B et al., 1989).

Bonmati et al. (1991) have reported that urease enzyme has the highest spatial variability followed by phosphatase in 1 year air dried soil samples of a 5 year old legume meadows in Pisa, Italy.

Studies on the depth-wise distribution of enzyme activities revealed a decreasing trend of dehydrogenase, urease and acid phosphatase activities with increase in soil depth in a hilly sandy loam profile of north-eastern India (Tiwari, 1996a). His study demonstrated persistent activities of these enzymes to a depth of 2m in sandy loam soil profile.

Tiwari (1996b) investigated the relationship between enzyme activities, microbial populations and soil respiration in some Indian soils namely, grassland, garden, orchard, fallow and arable soils of north-eastern hill regions. Multiple regression and simple correlation analysis of the studied parameters revealed widest range (40 fold) in urease enzyme activity for various soils whereas the narrowest range (1-15 fold) was recorded for the phosphatase activity. The dehydrogenase activity falls in the range of variation between the two enzymes, urease and phosphatase. The results showed that fungal biomass accounted largely for the variability in dehydrogenase, urease and phosphatase activities.

Marjadori et al. (1996) reported the influence of lead (Pb) pollution on two enzyme activities, soil dehydrogenase and phosphatase in four soils of south western Sardinia, Italy. They noted marked influence of lead (Pb) and
soil moisture on the activities of the dehydrogenase and phosphatase but the effect of variation in soil moisture was less for phosphatase activity. They further recorded reduction in activity of these enzymes at very high concentration of lead (i.e. 6000 µg Pb g⁻¹ soil) otherwise the doses did not result in clear fluctuations.

Kumari and Charya (1997) found significant positive correlation between soil enzyme activities and microbial population number in four polluted sites of Warangal, Andhra Pradesh, India. They found increased microbial colonies showing increased accumulation of soil enzymes. Positive correlation was observed between enzyme activities and soil nutrients such as nitrates, potassium and organic matter whereas iron and aluminum contents showed negative correlation.

Nagaraja et al. (1997) studied the effects of three pesticide applications on the enzymatic activities of dehydrogenase, phosphatase and urease in three different soils of Karnataka, India. They noted inhibition of the enzymes by pesticides in the order of captan > atrajin > aldrin at all concentrations of treatment. The 10 ppm aldrin treatment had no significant impact. Higher inhibitions of the enzyme activities were observed at 100 and 500 ppm concentrations of all pesticides.

Influence of compost addition and inorganic fertilizer treatment on soil biological and yield of crop under a cereal-legume on a typic Haplaustert increased dehydrogenase and alkaline phosphatase activities with addition of organic material whereas no significant influence of inorganic fertilizer treatment was observed (Manna and Ganguli, 1997). They further noted
significant correlation between the crop yield and activities of enzymes in soils.

Rao et al. (1997) reported increased activities of dehydrogenase, phosphatase and nitrogenase enzymes in soils with ley farming system in comparison to soils under conventional cultivation farming system in Jodhpur, India. Further, they recorded decreased enzyme activities in the ley and conventional farming (CF) systems with the increase in soil depth. Organic matter content and soil moisture were found to be the prime factors responsible for variation in enzyme activities.

Tiwari and Sharma (1998) recorded increased activities of dehydrogenase and urease soil enzymes with increased altitude up to 1100 masl in two mountain ranges of Arunachal Pradesh, north-eastern India. Correlation coefficient values of the enzyme activities and other soil properties revealed that the soil organic matter content was important factor that regulates the enzyme activities in highland soils.

Naseby et al. (1998) investigated the ecological impacts of the biocontrol agent, Pseudomonas fluorescens F113 in the rhizosphere of field-grown sugar beet using soil enzymes, acid and alkaline phosphatase, phosphodiesterase and arylsulphatase. They observed significant correlation between these enzyme activities in the rhizosphere highlighting the usefulness of enzyme assays to document variation in soil nutrient cycling.

Staddon et al. (1998) described the consequence of acid and alkaline phosphatase and arylsulphatase activities in soils from a jack pine (Pinus banksiana Lamb.) ecosystem after clear cutting, prescribed burning and
scarification. They recorded lower enzyme activity after prescribed burning in organic layers as compared to other treatments. They further noted inverse relationship of the acid phosphatase to soil pH and suggested that this enzyme assay may be useful for assessing the impact of fire on soil.

Higher activities of dehydrogenase and alkaline phosphatase enzymes were observed in treatments with tree-crop combination than in the treatment without tree in a 12 year old Dalbergia sisoo plantations (Chander et al., 1998). Their study showed that adoption of agroforestry plantation led to improved organic matter status of the soil, which is also reflected in the increased nutrient pool and microbial activities necessary for long-term productivity of the soil.

In an effort to assess soil quality using microbiological and biochemical procedures, Filip (1998) revealed that dehydrogenase activity measurement in soil samples affected by natural and anthropogenic activities may respond as one of the suitable indicators of soil quality. He has demonstrated that the dehydrogenase enzyme activity sensitively indicated the enhanced concentration of lead (Pb) in soddy-podzolic soil.

Gostkwoska et al. (1998) investigated the suitability of some biochemical and microbiological parameters for the evaluation of the degree of degradation in podzolic soils in the background of its differentiated usage in Lublin, Poland. Their study revealed that microbiological and biochemical changes in soil were more significant than that in chemical status of the soil. Further, they noted much stronger variation of the biochemical activity
(enzyme levels) of the layers of Ap horizon than physical and chemical properties of the studied soils.

Highly sensitive nature of soil biological and biochemical properties to environment stress suggesting their suitability to use in assessment of soil quality have also been reported by Trasar-Cepeda et al. (1998). They have demonstrated in their study that a balance existed between the organic matter content of a high-quality native soil and its biochemical and biological properties. Variations in the biochemical quality of a soil may disrupt this balance, in which case the equation may be useful as a biochemical quality index for soils.

Bendick and Dick (1999) studied the effects of field management on soil enzyme activities in vegetable crop rotation plots (VRP) and residue utilization plot (RUP) in Oregon. They observed significant treatment effects on the enzyme activities of the two sites ($P<0.05$). Enzyme activities (except $\alpha$-and $\beta$-glucosidase and $\alpha$-and $\beta$-galactosidase) were generally higher in centrinum grass fields than in cultivated fields. Their study revealed the growing recognition for the need to develop sensitive indicators of soil quality that reflect the effects of land management on soil and assist land managers in promoting long-term sustainability of terrestrial ecosystems.

Tiwari (1999) reported significantly greater ($P<0.05$) activities of dehydrogenase, urease and acid phosphatase in plots treated with organic manure or with N and P or a combination of both than in the control plots. However, he observed no significant impact of individual treatments of the fertilizer, C, N and P on the activities of these soil enzymes.
Palma et al. (2000) stressed the importance of biochemical properties of soil (particularly enzyme activity) as potential indicators of disturbances. Based on the result of their study in two different tillage systems (conventional and non-conventional) and two crop rotations (continuous corn and soybean-corn) it was concluded that enzymatic activities did reflect changes due to management and were suggested as sensitive indicators to different treatments.

**Dehydrogenase activity**

Dehydrogenase is an extracellular enzyme in the soil and considered to play an important role in the initial stages of the oxidation of soil organic matter by transferring hydrogen or electron from substrates to acceptors (Ross, 1971). Because of its importance in the organic matter transformation processes and its potential to indicate the available microbiological activity in the soil, dehydrogenase has been the subject of chosen biochemical tool in various fields of agricultural and soil science investigations.

Studies on effect of freezing and thawing of some grassland top soils on dehydrogenase activity revealed that storage of soil in frozen condition is useful for minimizing changes in some biochemical activities but it may sometimes result in increased activity when thawed samples are subsequently assayed (Ross, 1970 and 1972). It was concluded that a prolonged thawing period appears to be less essential for estimating dehydrogenase activity, particularly if anaerobic assay conditions are employed.
Reddy and Faza (1989) examined the enzymatic activity of dehydrogenase in sludge amended soil at different incubation periods. Their result indicated significantly inhibition of dehydrogenase activity at 24, 48, 96 h at all concentrations of sludge (40, 80 and 120 ton h\(^{-1}\)) treatments. The highest dehydrogenase activity in control (no sludge) soils was followed by 40, 80 and 120 ton sludge h\(^{-1}\) in decreasing order. The lower dehydrogenase activity in the sludge amended soils at all samplings could be due to the heavy metal concentration in sewage sludge (Reddy et al., 1987).

Brezezenska et al. (1998) investigated relationship between soil oxygen status and dehydrogenase activity in soils of Lublin, Poland. They noted increased activity of dehydrogenase activity with increase of soil water content and the conditioning temperatures. A combined effect of flooding and temperature to 30\(^{\circ}\)C caused an increased dehydrogenase activity on an average of 129 fold as compared with 15.9 KPa at 10\(^{\circ}\)C treatment. They suggested that soil water content and temperature influence the dehydrogenase activity indirectly by affecting the soil oxidation-reduction status.

Camina et al. (1998) measured dehydrogenase activity of acid forest soils rich in organic matter content of Galicia, N.W. Spain and revealed lower activity due to adsorption of formazan. They have suggested use of DMF-Ethanol and reference standards containing soils for determination of dehydrogenase activity at an enhanced recovery of formazan in acid soils rich in organic matter.
Phosphatase activity

Phosphatase activity is essential for conversion of organic substrates containing phosphorus into inorganic form through hydrolysis in the soil. Phosphatase being an important enzyme in soil is an oxidoreductase which plays a key role in P-cycle of the environment.

Since the development of an easy and simple method of assaying phosphatase activity in soil systems by using p-nitophenyl phosphate (Tabatabai and Bremner, 1969) as the substrate of phosphorus hydrolysis in laboratory conditions have brought the research in this field to an emerging field of soil enzymology.

Trasar-Cepeda and Gil-Sotres (1987) studied phosphatase activity of acid soils with high organic matter content in forest soils. They found higher activity of acid phosphatase between pH 5 and 6, which appeared to depend on organic activity of soil suggesting that enzymes originating from litter was progressively inhibited as it penetrated the soil.

Fox and Commerford (1992) examined the acid phosphatase activity in the rhizosphere of slashpine (Pinus elliottii) growing in A and Bh horizons of soils from two forested Spodosols. Their results indicated significantly high acid phosphatase activity in the rhizosphere of the Leon A and Bh horizons and Pomona Bh horizon soils. Further they noted decrease in phosphatase activity following application of phosphorus fertilizers.

Deng and Tabatabai (1997) observed significant affects of tillage management and crop residues placement on phosphatase activities. They noted higher activities of phosphatase in soils with no till x double mulch of
corn residues than other treatments. They also recorded significant correlation between phosphatase activities with organic C of the 40 soil samples tested suggesting that organic matter plays an important role in protecting and maintaining soil enzymes in their active forms.

Hysek and Sarapatka (1998) investigated the relationship between phosphatase active bacteria and phosphatase activities in forest soils in Izera Mountains of Czech Republic. The study reported that the number of acid phosphatase active colonies correlated positively with the number of alkaline phosphatase active colonies in F-A01 horizon and there was a high, positive correlation between the former and the level of ammonification in the H-AO2 horizon. It was shown that positive correlation between the number of alkaline phosphatase active colonies with organic carbon, the number of ammonification bacteria, and the number of mycomycetes in H-AO2 horizon. Neither acid nor alkaline phosphatase activities correlated with the number of phosphatase active colonies of bacteria.

**Urease activity**

Urease is a hydrolase enzyme responsible for hydrolytic conversion of the substrate, urea into carbon dioxide and ammonia. The urease enzyme assay is important in understanding mineralization process of N element and its response to the application of inorganic fertilizers, land use systems, tillage and soil management systems particularly its relationship to the agricultural practices has led to the extensive research investigation in the last three decades. As a result, the urease enzyme assay has become an important and routine practice in agricultural systems.
Klein and Kloths (1980) reported higher activities of urease in the no tillage grain-plots in comparison to the other plots. They also noted higher values of urease activity related to moisture and organic matter content of the soils in the order no tillage and tillage practices respectively.

Stott and Hagedon (1980) examined the interrelationship between selected soil characteristics and urease activities under two forest vegetations one native grassland and three clover/grass pastures in Benton County, Oregon. Highly positive correlation was observed between urease activity and soil organic matter ($r=0.59$). Principal component analysis demonstrated that urease activity, which when combined with four other factors, accounted for 65.5% of the observed variables in urease activity. Seasonal fluctuation in the urease activity was recorded where fluctuations in the activity levels were related to moisture and temperature conditions of the soils.

Vlek et al. (1980) reported fate of urea application under flooded conditions revealing approximately half of the urea incorporated into flooded water. This urea was hydrolysed largely at the soil-water interface and subsequently returns to the flood water (>80%) or is retained by the soil (<20%). They concluded that the fraction returning to flooded water is either taken by algae or volatilized.

Nor (1982) studied the activity and kinetic properties of urease in several Malaysian soils. He noted a significant correlation between $K_{\text{max}}$ and $V_{\text{max}}$ of urease activities. He also observed inhibition of urease activity very effectively with the use of $\text{Ag}^{3+}$ while $\text{Cu}^{2+}$ was only effective in two soils and
marginally effective in other soils. It was concluded that urease inhibitors have potential applications in reducing volatilization losses of ammonia derived from urea added to soils.

Investigations on the effects of temperature and moisture on urease activity in semi-arid tropical soils revealed that urease activity increased with increase in temperature from 10°C to 60°C (Vertisol) and 70°C (Alfisol). Further increase in the temperature decreased urease activity and completely inhibited at 100°C (Sahrawat, 1984). He also noted increase in urease activity with the increased moisture content up to field capacity beyond which activity declined.

O'Toole et al. (1985) studied urease activities in pasture and tillage soils of 10 soil series each. The results indicated higher activities of urease in pasture soils than in tillage soils in 8 of the 10 soil series. They recommended best prediction use of urease activity in grouping of agricultural soils by land use.

Palma and Conti (1990) studied the effects of various treatments of sample and seasonal variation upon urease activities on surface samples of Argentine Agricultural soils. They noted highest urease activity during summer and lowest during winter. The variation in the urease activity in different soil types under different vegetation revealed the activity of urease enzyme is related to the type of vegetation.

Tiwari and Mishra (1995) studied seasonal variation in urease activity in hilly soils under grassland and forests of north-eastern India. Their results
showed higher activity of urease under forest and grassland during rainy summer season and lower during winter season.

Studies on nitrification potential of urease in a mineral sub soil revealed the possible contribution of ammonia oxidizers to a complete hydrolysis of urea (Swenson and Bakken, 1998).

Klose and Tabatabai (1999) studied the relationship between urease and microbial biomass C and N and revealed highly significant relationship between urease activity and microbial biomass C and N. It was noted that urease activity of the microbial biomass, expressed as per cent of total urease activity ranged from 37.1 to 73.1% and the remaining 26.9 to 62.9% was extracellular.

6.3. METHODOLOGY

Enzyme activities

Dehydrogenase enzyme activity was assayed using modified 2,3,5-triphenyl tetrazolium chloride (TTC) reduction technique (Casida, 1977). Five grams of soil was placed in a test tube (15 x 2cm) and carefully mixed with 0.1g of CaCO₃ and 1.5 ml of distilled water added into the mixture. Then, 1 ml of 1% TTC solution was added and the tubes were incubated at 30°C for 24 h after plugging with cotton. The resulting slurry was transferred on Whatman No.1 filter paper and triphenyl formazan (TPF) was extracted with successive aliquots of concentrated methanol in a 50 ml volumetric flask. The extinction of the pink colour was read out with the help of spectrophotometer (Systronics-106) at 485 nm using methanol as control (without soil).
Dehydrogenase activity \((\mu g \ TPF \ g^{-1} \ dry \ soil \ 24 \ h^{-1}) = \frac{C \times 50}{W}\)

{Where, \(C = \) corrected reading of \(\mu g \ TPF \ ml^{-1}\) from the standard curve; \(50 = \) Extractant volume (ml); \(W = \) dry weight of soil}

Acid phosphatase activity was measured by \(p\)-nitrophenyl phosphate \((p\text{-NPP})\) reduction method of Tabatabai and Bremner (1969). 0.1g fresh soil sample was taken in a 50ml conical flask and mixed with 4 ml of modified universal buffer (MUB pH-6.5), 0.25ml toluene and 1ml of 0.115 M \(p\text{-NP}\) solution. The flask was swirled for a few seconds and plugged with cotton stopper and incubated for 1 h at 37°C. Then 1ml of 0.5 \(CaCl_2\) and 4ml of 0.5 M \(NaOH\) solutions were added simultaneously into the mixture before transferring into Whatman No.12 filter paper. The yellow coloured filtrate of \(p\)-nitrophenol phosphate (phosphoric acid) was read out with the help of spectrophotometer at 420 nm. For the control, 1ml \(p\text{-NPP}\) was added after \(CaCl_2\) and \(NaOH\) were added into the mixture without soil just before filtration.

Acid phosphatase activity \((\mu g \ p\text{-NPP} \ g^{-1} \ dry \ soil \ h^{-1}) = \frac{C \times 10}{W}\)

{Where, \(C = \) corrected reading of \(\mu g \ p\text{-NPP} \ ml^{-1}\) from the standard curve; \(10 = \) Solution volume (ml); \(W = \) dry weight of soil}

The urease activity was determined by urea reduction method of McGarity and Myers (1967). 10 g of fresh soil was placed in a 100 ml volumetric flask and treated with 1 ml of toluene, 10 ml buffer (pH-7) and 5 ml of 10% urea solution (freshly prepared). After a thorough mixing the flask was incubated for 3 h at 37°C in dark. For the control, 5 ml of 10% urea solution was replaced by 5 ml of sterile distilled water. After incubation the
volume of the flask was made up to 100 ml with distilled water and shaken thoroughly and transferred the filtrate through Whatman No.5 filter paper. The ammonia released as a result of urease activity was measured by indophenol blue method. 0.5 ml of the filtrate was taken into a 25 ml volumetric flask and 5 ml of distilled water was added. Then 2 ml of phenolate solution (mixture of 20 ml of stock A (62.5 g phenol crystals dissolved in a minimum volume of methanol and made up the volume up to 100 ml with ethyl alcohol after adding 18.5 ml acetone) and 20 ml of stock B (27 g NaOH dissolved in 100 ml distilled water and kept in freezer) was added. Thereafter, 1.5 ml of sodium hypochlorite solution was added. The final volume of the flask was increased up to 25 ml with distilled water and the blue colour was read out with the spectrophotometer at 630 nm.

Urease activity \((\text{mg NH}_4^+\text{-N g}^{-1} \text{ dry soil 3 h}^{-1}) = \frac{C \times 25 \times 100}{W}\) 

\(\text{Where, } C = \text{corrected reading of mg NH}_4^+\text{-N ml}^{-1} \text{ from the standard curve; 25= Extractant volume (ml); 100= Total solution volume; W= dry weight of soil}\)

Data reported anywhere in the text, table and figures for three enzymes (Dehydrogenase, phosphatase and urease) are mean values of triplicate analyses.

6.4. RESULTS

Dehydrogenase activity

Activity of dehydrogenase enzyme ranged from 6-189\(\mu\)g TPF g\(^{-1}\) dry soil 24 h\(^{-1}\) in soils of degraded, moderately degraded and undegraded sites at surface and subsurface layers (Fig. 6.1). Maximum dehydrogenase activity was recorded from undegraded site (189\(\mu\)g TPF g\(^{-1}\) dry soil 24 h\(^{-1}\)) followed by moderately degraded site (145\(\mu\)g TPF g\(^{-1}\) dry soil 24 h\(^{-1}\)) while
Fig. 6.1. Dehydrogenase activity in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD, $P < 0.05$)
Fig. 6.2. Acid phosphatase activity in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD, $P<0.05$)
Fig. 6.3. Urease activity in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD, *P*<0.05)
Table 6.1. One-way analysis of variance (ANOVA) of the biochemical characteristics of soil in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers at $P<0.05$.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Source of variation</th>
<th>Surface layer</th>
<th>Subsurface layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-ratio</td>
<td>P-level</td>
<td>F-ratio</td>
</tr>
<tr>
<td>Dehydrogenase activity</td>
<td>DFxMDFxUDF</td>
<td>12.334</td>
<td>2.63 x 10^{-5}</td>
</tr>
<tr>
<td></td>
<td>DFxMDF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MDF x UDF</td>
<td>8.573</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>DF x UDF</td>
<td>22.749</td>
<td>1.89 x 10^{-5}</td>
</tr>
<tr>
<td>Phosphatase activity</td>
<td>DFxMDFxUDF</td>
<td>8.579</td>
<td>4.71 x 10^{-4}</td>
</tr>
<tr>
<td></td>
<td>DFxMDF</td>
<td>6.816</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>MDF x UDF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DF x UDF</td>
<td>14.2766</td>
<td>4.52 x 10^{-4}</td>
</tr>
<tr>
<td>Urease activity</td>
<td>DFxMDFxUDF</td>
<td>3.281</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>DFxMDF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MDF x UDF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DF x UDF</td>
<td>5.599</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Note: Insignificant values are denoted by "-" sign.

Table 6.2. One-way analysis of variance (ANOVA) of the biochemical characteristics of soil between surface and subsurface soil layers in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites ($P<0.05$).

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Study sites</th>
<th>F-ratio</th>
<th>P-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrogenase activity</td>
<td>DF</td>
<td>26.891</td>
<td>4.702 x 10^{-6}</td>
</tr>
<tr>
<td></td>
<td>MDF</td>
<td>24.616</td>
<td>1.000 x 10^{-5}</td>
</tr>
<tr>
<td></td>
<td>UDF</td>
<td>27.489</td>
<td>3.872 x 10^{-6}</td>
</tr>
<tr>
<td>Phosphatase activity</td>
<td>DF</td>
<td>17.4788</td>
<td>1.288 x 10^{-4}</td>
</tr>
<tr>
<td></td>
<td>MDF</td>
<td>15.274</td>
<td>3.034 x 10^{-4}</td>
</tr>
<tr>
<td></td>
<td>UDF</td>
<td>10.89</td>
<td>0.002</td>
</tr>
<tr>
<td>Urease activity</td>
<td>DF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MDF</td>
<td>8.349</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>UDF</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Insignificant values are denoted by "-" sign.
the minimum was recorded from the degraded site (14μg TPFg⁻¹ dry soil 24 h⁻¹) at both the soil depths. The maximum activity was recorded in the month of January 2000 from the undegraded site while the minimum was recorded in the month of April 1999 from the degraded site at surface soil layer. However, the subsurface soil layer contained maximum dehydrogenase activity in undegraded site in the month of January 2000 and minimum in degraded and moderately degraded sites in the month of July 1998. In general, the subsurface layer had higher dehydrogenase activity in all the sites and at both the soil layers.

There was a marked seasonal variation of dehydrogenase enzyme activity in all sites and at both the soil depths (Fig. 6.1). The highest activity was recorded during winter dry months (November to January) and lowest in the late spring and early rainy months (February–April). The variation in dehydrogenase activity among the three sites varied significantly (P<0.05) at surface and subsurface soil layers except between the degraded and moderately degraded sites in which the variation in dehydrogenase activity was insignificant (Table 6.1). Similarly the variation in dehydrogenase activity of degraded, moderately degraded and undegraded sites between the two soil depths were also significant at P<0.05 (Table 6.2) Dehydrogenase activity was positively correlated (P<0.05) with soil pH and organic C in all sites (Table 3.3 and 3.4). Similarly it was also related to phosphatase activity at subsurface layer of undegraded forest site.
Acid phosphatase activity

Acid phosphatase activity of the soil at both the surface and subsurface soil layers of all study sites varied from 13μg p-NPP g\(^{-1}\) dry soil h\(^{-1}\) to 180μg p-NPP g\(^{-1}\) dry soil h\(^{-1}\) at both the surface and subsurface soil layers (Fig. 6.2). The undegraded site contained maximum acid phosphatase activity followed by moderately degraded site while the minimum was recorded from degraded site at surface and subsurface soil layers. Generally, surface soil layer contained higher acid phosphatase activity than the subsurface soil layers in all the sites.

The seasonal variation in acid phosphatase activity showed three peaks during the entire period of two years in the three study sites. The highest peak was recorded in the month of February 2000 followed by January and September 1999. The results showed higher acid phosphatase activity during the intermediate period between late winter and early spring seasons and between, late rainy and early winter i.e. at the end of rainfall and winter season the activity was maximum at both soil layers.

The variation in acid phosphatase activity among degraded, moderately degraded and undegraded forest sites were significant \((P<0.05)\) at both soil depths (Table 6.1). The variation in acid phosphatase activity was also significant between the two soil depths of degraded, moderately degraded and undegraded forest sites (Table 6.2). Phosphatase activity was positively correlated \((P<0.05)\) with organic C in all study sites at both soil layers (Table 3.3 and 3.4). Microbial biomass N was also related positively
with phosphatase activity in the surface soil layers of degraded, moderately degraded and undegraded forest sites.

**Urease activity**

Urease activity varied from the minimum of 0.3 mg NH$_4^+$-N 100 g$^{-1}$ soil 3 h$^{-1}$ in the degraded site to a maximum of 5.81 mg NH$_4^+$-N 100 g$^{-1}$ soil 3 h$^{-1}$ in the undegraded site (Fig. 6.3). The maximum urease activity occurred in the month of August 1998 at both the soil layers of undegraded site whereas the minimum was recorded from the degraded site in June 2000 from the surface layer and in May 1999 from subsurface layer respectively.

There was significant variation in urease activity distribution among the three sites and between the degraded site and undegraded site at surface soil layer only (Table 6.1). However, no significant variation was observed between the degraded and moderately degraded sites and between moderately degraded and undegraded sites at both the surface and subsurface soil layers. The variation in urease activity was significant ($P<0.05$) between the surface and subsurface soil layers of moderately degraded site only but no significant variation of urease activity was recorded from the degraded site and undegraded sites (Table 6.2). Urease activity was positively related to soil pH of undegraded site at surface layer and with available-N in degraded and moderately degraded sites at subsurface layers (Table 3.3 and 3.4).
6.5. DISCUSSION

6.5.1. Spatial variation of dehydrogenase, acid phosphatase and urease enzyme activities

Dehydrogenase enzyme activity was significantly higher in the soil of undegraded forest site in comparison to the degraded and undegraded sites. The reason for higher dehydrogenase enzyme activity could be due to the presence of higher organic matter on the forest floor and abundant tree cover in this site providing favourable microclimatic conditions for larger microbial growth and accumulation of more enzymes. Tiwari et al. (2002) have reported higher dehydrogenase activity in an undisturbed forest site in comparison to a degraded site and a slightly degraded site in humid tropical regions of north-eastern India. They have noted 40% and 25% reduction in dehydrogenase activity of degraded and moderately degraded sites at surface and subsurface soil layers in comparison to the undisturbed site. The decline in dehydrogenase activity in the degraded site in the present study reveals the long-term detrimental effect of shifting cultivation practice and selective logging of forest trees on biochemical characteristics of soil. The removal of the vegetation by clear-cutting prior to the showing of crops and continuous cutting of selected trees caused significant reduction in dehydrogenase activity and other microbiological properties in soils of these sites. Garcia et al. (1997) found that devegetation of soils in semi-arid areas lead to reduction of their biochemical quality in contrast to natural or undisturbed area. As an indication of microbiological metabolic activity, dehydrogenase activity was significantly affected as a result of devegetation in their study.
Acid phosphatase activity was higher in the undegraded forest site than degraded and moderately degraded sites at both the surface and subsurface soil layers. The undegraded site had highest acid phosphatase activity due to the presence of higher organic matter on the forest floor which influenced greater growth of microorganisms and accumulation of more soil enzymes (Dinesh et al., 1998). Another reason could be the favourable soil reaction in the undegraded site which influenced higher enzyme activity and declined with increased soil acidity. Staddon et al. (1998) also reported similar correlation of acid phosphatase activity with soil pH in clear-cut, burned and scarified jackpine (*Pinus banksiana* L.) community soils. Increase in acid phosphatase activity with increase in soil pH have been reported in detailed by Dick et al. (1998). Similar results of higher acid phosphatase activity was observed in pineapple (*Annanus comosus* L.) orchard soils of different ages (Tiwari, 1988). His study revealed significant correlation between acid phosphatase activity and organic C content in the 1, 5 and 10 year old pineapple orchards.

Urease activity showed significant variation among soils of all the three study sites and at surface soil layers but there was no significant variation among the sites at subsurface soil layers (Table 6.1). However, urease activity, remained highest in the undegraded site during its peak activity periods than degraded and moderately degraded sites at both soil layers. This results suggest no detrimental effect of shifting cultivation and selective logging of trees in the forest soils on urease activity. Earlier reports on urease soil enzyme activity have revealed generally higher urease activity.
in older pineapple orchard soils than younger ones (Tiwari, 1988), in forest soils than in grasslands (Tiwari and Mishra, 1995), in pasture than in tillage soils (O'Toole et al., 1985) and in no tillage soils than in plow-grain soils (Klein and Kloths, 1980). Pancholy and Rice (1973) showed that urease activity is related to type of vegetation and the quality of incorporated organic materials in the soil. Similarly, Palma and Conti (1990) also reported significant variation in distribution of urease activity in grassland and forest soils revealing direct relationship between pattern of urease activity to the type of vegetation and impact of organic matter.

6.5.2. Seasonal variation of dehydrogenase, acid phosphatase and urease activities

Distribution of dehydrogenase activity was significantly affected by seasonal variation in the three study sites and at two soil depths. Dehydrogenase enzyme activity was higher during winter dry period and lower during summer rainy season. The peak of dehydrogenase enzyme activity occurred in the months of November 1998 and another in January 2000 where the soil was dry and temperature was also lower than the average. This suggests that accumulation of dehydrogenase enzyme takes place with the onset of winter season which is against the decreasing moisture gradient in all the three study sites and at two soil depths. Dormaar et al. (1984) also reported higher dehydrogenase activity during winter months and lower during summer months under two grazing regimes of two different study site. They did not find close relationship between dehydrogenase activity and soil organic matter in either site though the relationship existed between the two areas. However, they assumed that
hygrothermal conditions (soil moisture and temperature) were the overriding factors of dehydrogenase activity. The results in the present study indicated that higher dehydrogenase activity being controlled by presence of higher organic matter content during the dry winter period and favourable soil pH of the soils.

Acid phosphatase activity was significantly influenced by fluctuations in seasonal climatic variables in soils of degraded, moderately degraded and undegraded forest sites. The peaks of the acid phosphatase activity in the present study appeared in the intermediate period of winter and spring and another in rainy-winter period reveal that this soil enzyme activity is dependent on the moisture availability in all sites and at both the soil depths. However, the sharp decline in the acid phosphatase activity in the month of October in both the years 1998 and 1999 could not be explained despite its positive correlation with soil pH, organic C, total N and microbial biomass N. Higher phosphatase activity was also reported during spring-summer from pineapple orchard soil of north-eastern India (Tiwari, 1988 and Tiwari, S.C. et al., 1989ab) and during peak rainy seasons of acid soil rich in organic matter content in Galicia, NW Spain (Trasar-Cepeda and Gil-Sotres, 1987). However, the results of the present study corresponds to the findings of Dormaar et al. (1984) which reported higher acid phosphatase activity during winter months due to higher root biomass and microbial population in the soils.

There was no clear trend in variation of urease activity along seasonal gradients in all study sites though the activity. The monthly variation in
urease activity was insignificant in degraded, moderately degraded and undegraded sites at both soil layers. Therefore, seasonal variation in urease activity remained unchanged throughout the sampling period of the study.

6.5.3. Depth-wise variation of dehydrogenase, acid phosphatase and urease enzyme activities

In general, dehydrogenase activity was higher at the surface soil layers of all sites than the subsurface soil layers. This reveals that dehydrogenase enzyme is produced at the surface soil layer which contains larger amounts of decomposed litter and organic carbon. However, the lower dehydrogenase activity in the subsurface soil layer could be due to lower quantity of dehydrogenase enzyme produced in this layer due to the presence of lower organic matter and microbial populations. Higher dehydrogenase activity was reported from the surface soil layers which declined with increasing depth in pineapple orchard soils of north-eastern India (Tiwari et al., 1987a). The reason for higher dehydrogenase enzyme activity in the surface soil layer was due to presence of higher bacterial population, organic carbon content, favourable moisture content and temperature (Khan, 1970; Das, 1980; Dkhar and Mishra, 1983; Baruah and Mishra, 1984 and Tiwari et al., 1987b). Dehydrogenase enzymes appeared to be linked with microbial activity associated with initial breakdown of organic matter and are dependant on the metabolic state of the soil or on the biological activity of the microbial populations (Ross, 1970). The results in the present study reveals that more accumulation of dehydrogenase enzyme at the surface soil layer having higher microbial population and organic carbon content than at the subsurface layers of all study sites.
The activity of phosphatase was higher at the surface soil and lower at the subsurface soil layer of degraded, moderately degraded and undegraded forest sites. There was decrease in acid phosphatase activity of the soil with increase in the soil depth from highest at the surface to the lowest in the subsurface layer of all sites. This reveals presence of higher soil enzyme at the surface soil layer and lower enzyme accumulation in the subsurface soil layer. The presence of higher organic matter, pH and total N at the surface soil layer could be other important factors for higher acid phosphatase activity in the surface soil layer since these properties of soil were correlated positively to the acid phosphatase activity. Trasar-Cepeda and Gil-Sotres (1987) also reported decrease in soil enzyme activity of acid phosphatase with increase in the soil depth and its positive correlation with organic carbon content. This suggests that acid phosphatase enzyme originated mainly at the surface soil layer, which contained more litter and was progressively inhibited with increase in soil depth, which was associated with a decrease in organic C content. Their results also confirmed significant correlationship of phosphatase activity with the soil pH under different tillage and residue managed soils. Higher acid phosphatase was also reported from the pineapple orchard soils of north-eastern India (Tiwari, 1988; 1996ab and Tiwari, S.C. et al., 1989ab). Their results confirmed that enzyme activity of acid phosphatase decreased from surface organic layer to deeper subsurface layers along the decreasing trend of soil organic C, moisture and other inorganic nutrients and lower microbial populations.
Distribution of urease activity was not significantly different between the surface and subsurface soil layers of the degraded and undegraded sites. However, the urease activity varied significantly between two soil depths of moderately degraded site. Singh et al. (1995) also reported insignificant variation in the distribution of urease activity along the gradient of soil depth from surface to the deeper soil depths, which is against the earlier reports of the decreasing turnover of urease activity with increase in soil depth (Tiwari et al., 1987a and Tiwari, 1988 and 1996).