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Soil Enzymes Research: A Review

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Abstract: Enzymes are biologically produced proteinic substances having specific activation in which they combine with their substrates in such a stereoscopic position that they cause changes in the electronic configuration around certain susceptible bonds. Their significance in all spheres including soil, is worth tested and reported. In plant nutrition their role cannot besubstituted by any other substance and its function is quite pragmatic in solubilizing and dissolving the much needed food in ionic forms for the very survival of animal and plant kingdom. World over, innumerable researchers have contributed their efforts in exploring enzymes. This paper reviews some of the important factors affecting its behaviour, reactions in soil environment, correlation with other enzymes and soil properties, preceded by its historical perspective and sources of production.

Key words: soil, enzymes, factors, applications

Introduction

Nutrient cycling in soils involves biochemical, chemical and physiochemical reactions, with biochemical processes being mediated by microorganisms, plant roots, and soil animals. It is well known that all biochemical reactions are catalyzed by enzymes, which are protein with catalytic properties owing to their power of specific activation. Enzymes are catalysts, that is, they are substances that without undergoing permanent alteration cause chemical reactions to proceed at faster rates. In addition, they are specific for the types of chemical reactions in which they participate (Tabatabai, 1994). Enzymes specificity is often dictated by the nature of the groups attached to the susceptible bonds. e.g., maltase hydrolyzes maltose to glucose, but not vice versa. Differences between the two substances seem slight in that maltose is an α-glucoside and cellobiose is a β-glucoside. Both α and β-glucosidases are present in soils (Eivazi and Tabatabai, 1988). Physiochemical measurements indicate that enzyme-catalyzed reactions in soils have lower activation energies than non-enzyme catalyzed reactions and, therefore, have faster reaction rates (Browman and Tabatabai, 1978; Dick and Tabatabai, 1978). Enzymes in soil are similar to enzymes in other systems, in that their reaction rates are markedly dependent on pH, ionic strength, temperature, and the presence or absence of inhibitors (Burns, 1978 and Tabatabai, 1982).

The first landmark in the history of enzymology was the extraction of the enzymes from the yeast cells by Bucherer in 1897. He extracted from the yeast cells, a clear solution that was capable of catalyzing alcoholic fermentation of sugar. Bucherer called the mixture of organic catalysts "zymase".

In 1926, Sumner isolated urease in crystalline form from jack bean (*Canavalia ensiformis*) meal, for which he received a Nobel Prize (Dick and Tabatabai, 1993). Later between 1930-1936, he isolated pepsin, trypsin and chymotrypsin and confirmed the view that the enzymes were proteins (Khan, 1989). The progress in the soil enzymology was extremely slow until 1950, but exponential progress has been made in this field within the past half century. The history of abiontic soil enzyme research has been elegantly prepared by Skujins (1978). Therefore, soil may be looked on as a biological entity, that is, a living tissue (Quastel, 1946) with complex biochemical reactions.

Enzymes are produced from plants, animals and microorganisms, but production from the first two groups is limited for several reasons. Cultivation of plants is restricted to areas where climate is suitable. It is generally seasonal impeding steady enzyme production. As the concentration of enzymes in plant tissues is generally low, processing of large amounts of plant material is necessary. Spier and Ross (1978), in their review of soil phophatases, suggest that microorganisms seem the logical choice for supplying most of thesoil enzyme activity, because of their large biomass, high metabolic activity and relatively larger amount of extracellular enzymes than can plants or animals.

Ribonucleases and alkaline phosphatases are excreted by *Bacillus subtilis* under certain conditions (Cashel and Freese, 1964) and pyrophosphate and acid phosphatase may exist extracellularly on the surface of cell walls of *Saccharomyces mellis* (Weimberg and Orton, 1963, 1964).

Plants have been considered a source of extracellular enzymes in soil. Estermenn and McLaren (1961), using barley (*Hordeum vulgare*) as the test plant, found that root caps possessed phosphatase activity. Juma and Tabatabai (1988) demonstrated that sterile corn (*Zea mays*) and soybean (*Glycine max*) roots contain acid phosphatase, but no alkaline phosphatase activity. In other work, Juma (1976) demonstrated that sterile corn and soybean roots could exude phosphatases into a solution that surrounded them. Roots, placed into sterile buffer or water for 4-48 hr, released phosphatase into the solution. Greater amounts of phophotase were released into water than into the buffered solution. Enzymes synthesized by plants, added to soil as plant residues, may remain active. Phosphatase activity in soil has been observed to be associated with intact cell walls of plant tissues, with cell wall fragments and with amorphous organic material (Ladd, 1978). Enzyme activity is considerably greater in the rhizosphere of plants than in bulk soil, and this increased activity is due to either a specific flora or the plant root, or to both (Skujins, 1967). Ramirez-Martinez and Mclaren (1966) reported that the amount of phosphatase activity in one gram soil was equivalent to 10^{10} bacteria or one gram of fungal mycelia.

Many enzymes have been detected in soils but only a few assays have been evaluated thoroughly enough to be considered standard. According to Dick and Tabatabai (1993) many of the enzymes detected in soils are hydrolases (they catalyze the hydrolytic cleavage of chemical bonds), but other falls in the class of oxidoreductases (they catalyze oxidation-reduction reactions), transferases (they catalyze transfer of molecular substituents among molecules), and lyases (they catalyse the removal of groups from substrate molecules without hydrolysis).

Soil Enzymology: A review: The topic under review is elaborated in four categories;

Factors affecting enzyme activity
Enzymes and the soil environment
Correlations
Applications of enzymes in soil plant environment

Factors affecting enzyme activity: This section carries the

effect of different factors i.e. pH, temperature, depth, ionic strength and salinity etc. on enzyme activity.

Senwo and Tabatabai (1996) showed that soil aspartase has its optimum activity at buffer pH 8.5 and is inactivated at temperatures above 40°C. Preheating soil samples for two hours before assay of aspartase activity showed that the enzyme is stable upto 40°C in field-moist samples and upto 70°C in air-dried samples. Frankenberger and Tabatabai (1991a) revealed that optimal temperature for soils L-glutaminase (L-glutamine amidohydrolase, EC 3.5.1.2; catalyzes the hydrolysis of L-glutamine to produce ammonia and glutamic acid) activity was 50°C and denaturation began at 55°C. Among the various treatments that affected L-glutaminase activity in soils, autoclaving (121°C, 1hr), formaldehyde (1mL 5 g^{-1} soil), dimethyl sulfoxide (1mL 5g^{-1} soil) and Na (5m*M*) reduced the activity by 92, 96, 78 and 14%, respectively. L-Glutaminase activity was greater in toluene treated soils than in untreated soils. Frankenberger and Tabatabai (1991b) further concluded that the activity of L-glutaminase in soil profile samples decreased with sample depth. Cochran *et al.* (1989) revealed that tillage and residue treatments had no effect on biomass and dehydrogenase, urease and phosphatase activities while these were generally greater in the agricultural Ap horizon than in the A horizon of an adjacent black spruce forest. Staddon *et al.* (1998) found that clear cutting followed by burning of organic soils of a jack pine (*Pinus banksiana* L.) lowered the activities of acid phosphatase, alkaline phosphatase and arylsulfatase activities. Bonmati *et al.* (1991) evaluated the spatial variability of urease and phosphatase in a 5 year old grass legume association. Twenty four top soil samples (0-20cm) were collected from a 15x40 m meadow, air dried, sieved and then stored at room temperature for one year before being analyzed. Four different procedures of statistical analysis resulted that urease activity was the most variable whereas phosphatase and casein-hydrolysing activities showed a similar viability. In another study soil samples (top soils 0-20 cm to sub soils 20-40 cm) collected from five different land use systems i.e. primary forests, secondary forests, coffee plantation and cultivated plants; showed that the activities of phosphatases, betaglucosidase, and urease were significantly higher in topsoils and in most cases in the primary forests or in the secondary forests (Salam *et al.*, 1998). Gupta and Bhardwaj (1990) found that both phosphatase and urease activity was greater in grassland and forest soils than uncultivated soils, with urease activity being greatest in the grassland soils. Shukla *et al.* (1989) reported temporal and depthwise distribution of enzymes in three different farming systems on valley, terrace and sloping land for two crop cycles of potato. In general, microbial number, soil respiration and enzyme (dehydrogenase, urease and phosphatase) activity occurred in the order of valley land > terrace land > sloping land. Populations of fungi and bacteria, activities of dehydrogenase, urease and phosphatase enzymes were generally higher in 0-10 cm deep soil and decreased with depth. Spring-summer season showed maximum microbial population and enzyme activities than winter season (Tiwari *et al.*, 1989). Bopaiah in 1990 reported that the activity of urease, phosphatase and dehydrogenase enzymes at three soil depths in the root zone and interspaces of coconut were greater while in arecanut palm only urease and phosphatase activities were greater in the root zone soils. Iftikhar and Khan (1988) found that enzyme activity was decreased with increasing soil salinity (EC). The decline in enzyme activity (amylase, catalase, urease, phosphatase) with increasing salinity appeared to be associated with change in osmotic potential of the soil due to higher salt concentrations, specific ion toxicities and salting out effect of soluble salts on enzymes protein.

Saa *et al.* (1998) revealed the effects of laboratory incubation on P form distribution and phosphomonoesterase activity in a forest soil recently affected by a wildfire (B) compared with its effects on these properties of an unburnt soil from an adjacent plot (UB). Samples of surface (0-5 cm) and subsurface (5-10 cm) layers of both soils were incubated at 75% field capacity and 28 °C. After 0, 1, 2, 4, 6 and 11 weeks, samples were withdrawn and fractionated and the P contents of fractions from corresponding layers were compared. The 0-5 cm layer was most severely affected by the fire, which caused oxidation of organic P (P-o) and a marked decrease in the content of residual P (P-r). The major process occurring during the incubation of this layer was microbial immobilization of inorganic P (P-i) in organic forms. By contrast, the 5-10 cm layer behaved similarly to the UB soil during incubation: essentially, P-r was transformed into rapid turnover P-o ($NaHCO_3$-extractable P-o + NaOH-extractable P-o), and P-i was occluded. After destruction of the enzyme due to burning, both layers of the B soil exhibited a very low initial rate of phosphomonoesterase activity. The failure of the enzyme activity to increase during incubation coincided with high labile P-i concentrations, suggesting that microbial synthesis of new phosphomonoesterase enzyme was repressed.

In another study acid phosphatase and alkaline phosphatase active colonies of bacteria, isolated from forest soils, were stained. The activity of acid and alkaline phosphatase and other soil properties (the number of aerobic bacteria, basal respiration, the level of ammonification, the number of bacteria active in ammonification, the level of nitrification, the number of micromycetes) were compared with the number of bacteria belonging to the genus *Micrococcus*. Soil samples were taken from F-AO1 (fermentative), H-AO2 (humic), and A (basic) horizons. The soil samples were taken from beneath forest stands in the Izera Mountains (North Bohemia, Czech Republic). The number of acid phosphatase active colonies correlated positively with the number of alkaline phosphatase active colonies in the F-AO1 horizon, and there was a high positive correlation between the former and the level of ammonification in the H-AO2 horizon. The number of alkaline phosphatase active colonies correlated positively with organic carbon, the number of ammonification bacteria, and the number of micromycetes in the H-AO2 horizon. The A horizon was almost biologically inactive. Neither acid nor alkaline phosphatase activities correlated positively with the number of phosphatase active colonies of bacteria (Hysek and Sarapatka, 1998).

Kramer and Green (2000) reported that the seasonal dynamics of acid and alkaline phosphatase activity (mu g p-nitrophenol released g^{-1} soil h^{-1}), soil water potential and temperature, and the relationship of phosphatase activity to plant and soil microbial processes underneath *Juniperus monosperma* canopies and *Hilaria jamesii* dominated intercanopy areas were studied in a northern Arizona pinyon-juniper ecosystem. Alkaline phosphatase activity was significantly higher in soils under junipers (126.5 +/- 3.9 mu g p-nitrophenol g^{-1} soil h^{-1}) than in intercanopy soils (106.6 +/- 4.0 mu g p-nitrophenol g^{-1} soil h^{-1}), and significantly exceeded acid phosphatase activity by a factor of 6. Seasonal high phosphatase activities were up to 2.4 times greater than seasonal lows. Activities were maximal in summer and winter. Juniper soils were cooler than intercanopy soils except during the coldest months of the year, when they were up to 2.7 °C warmer. Intercanopy soils were up to 6.2 °C warmer than juniper soils, and had the highest (30.0 +/- 0.3 °C) and the lowest average temperatures (2.3 +/- 0.2 °C). Soil microclimate explained as much as 20% of the variation in acid and alkaline phosphatase. Temperature and water potential together were better predictors of phosphatase activity than either one alone. The soil water potential class -0.1 MPa greater than or equal to psi > 0.5 MPa was the most frequent best predictor of phosphatase activity, especially alkaline phosphatase. The winter peak in alkaline phosphatase activity is attributed to a

build up of phosphatase released into the soil from dying soil organisms, and the desorption and reactivation of previously accumulated phosphatase.

Changes in the activities of soil enzymes (acid and alkaline phosphatases, beta-glucosidase, and arylsulfatase) associated with continuous cultivation of cassava, sugarcane, and pineapple were studied in middle terrace areas of Lampung Province, South Sumatra, Indonesia. Soil samples were collected from fields continuously cultivated with cassava for the period ranging from 0 to 10 y, with sugarcane from 0 to 20 y, and with pineapple from 0 to 10 y. Continuous cultivation did not show conspicuous effects on soil pH, and contents of total N, organic C, and available P as well as soil enzymatic activities. However, the changes in the activities of the soil enzymes generally showed a significant relationship with the contents of soil organic C and total N (Salam *et al.*, 1999).

Neitzke (1999) reported that microbiological properties along four transects from farmland to calcareous grasslands were investigated. It was examined whether changes in microbiological properties had developed in the peripheral zone of the calcareous grasslands parallel to the change in species composition caused by nutrient input. At three study sites the enzyme activities in the soils of farmland were lower than in the soils of peripheral zones and the calcareous grasslands. At two study sites basal respiration was also lower in the farmland soils. Two transects showed differences between the soils of the peripheral zone and the calcareous grassland. At the Galgenberg basal respiration and enzyme activities in the peripheral zone were lower than in the calcareous grassland, which might be due to a decrease in root mass and an increase in phosphate contents (inhibition of phosphatase activity). In the peripheral zone of Forstberg site increased microbial biomass was found, caused by higher production and favorable C/N ratios of the litter.

Enzymes and the soil environment: Senwo and Tabatabai (1999) conducted a research on the activity of aspartase activity in soils, effects of trace elements and its relation-ships to other amidohydrolases. The enzyme aspartase (L-aspartase ammonia-lyase, EC 4.3.1.1) catalyzes the hydrolysis of L-aspartate to produce fumarate and NH_3. At 5 μmol g^{-1} soil, all the trace elements inhibited aspartase activity in the three field moist soils and their air dried counterpart. With most of the elements, greater inhibition was found in air-dried than in field-moist soils. Frankenberger and Tabatabai (1991a) revealed that most of the 21 trace elements, 12 herbicides, 2 fungicides and 2 insecticides studied inhibited the activity of L-glutaminase, but the degree of inhibition varied among the soils. Kim and Hong (1988) reported that herbicides inhibited the activities of soil enzymes in the early stage of treatment but increased the activities of urease, L-glutaminase and protease later. In another study it was found that absolute urease and phosphatase activities decreased during composting, showing a minimum at 91 and 65 days, respectively, thereafter they increased slightly (Garcia *et al.*, 1992). Perucci (1990) revealed that when municipals solid waste compost were added in soil, alkaline phosphomonoesterase, phosphodiesterase and deaminase activity remained constant after reaching maximum values (3-5 months). Arylsulphatase, urease and protease activity returned to baseline after reaching a maximum (2-3 months). Guan (1989) found that application of wheat and maize straw as manure increased the invertase activity of the soil by 40-91 and 150-156 times, respectively, compared with control. The application of compost, pig, horse and cattle manure also increased the activity of invertase, but to a lesser extent. The activities of urease and alkaline phosphatase were also increased by the application of the manures. Grejtovsky (1991) revealed that 3 or 4 years after incorporation of fly ash (500 t/ha), activity of soil phosphatase was significantly inhibited. Pig slurry stimulated phosphatase and urease activity, but decreased phosphatase activity in soils under irrigation. Shendzhen *et al.* (1991) investigated that urease activity was stimulated by Mo and Co, initially inhibited but later stimulated by B and Cu and depressed by Mn and Zn. Phosphatase activity, though not great, was beneficially influenced by both NPK and trace elements, with significant increase in mineralization of organic P compounds.

Wick *et al*. (1998) reported that soil microbiological and soil biochemical parameters (pH, exchangeable basic cations, inorganic and organic phosphorus pools, total organic carbon and total nitrogen, microbial biomass carbon, acid and alkaline phosphatase, beta-glucosidase and protease activity) of soil were identified as indicators of soil quality under improved fallow management systems with senna, leucaena and pueraria on severely degraded and non-degraded soil. Principal component analysis demonstrated that soil organic matter related nutrient dynamics was the major contributor to explain the total variance (>80%) of the sites under the prevailing experimental conditions. Highest loadings with the major principal component were provided by microbial biomass, alkaline phosphatase, total N, beta-glucosidase and organic C. Contrasting fallow management systems (alley cropping, live mulch, planted fallow, controls in long-term experiments) at three sites differing in degree of soil degradation could be evaluated adequately by these indicators. β-Glucosidase indicated soil quality changes better than total organic carbon. Alkaline phosphatase was more sensitive than microbial biomass in characterizing the effects of improved fallow management on site degradation. Acid phosphatase and protease were not considered sensitive indicators for soil quality evaluations of these long-term management trials. Pueraria sustained soil quality on the non-degraded site but did not improve the severely degraded site, suggesting that pueraria is a soil fertility maintenance crop. In contrast, senna improved the degraded sites and more so on the most severely degraded site. Apparently, senna can be considered a suitable candidate for soil restoration purposes.

Parthasarathi and Ranganathan (2000) reported cellulase, amylase, invertase, protease and phosphatase activities in pressmud (PM, filter cake) and PM vermicasts of fresh, 15- and 30-day-old casts of *Lampito mauritii* and *Eudrilus eugeniae* decreased considerably with cast age. Enhancement of the activities of these enzymes could be ascribed to the nutrient-rich substrate, active microbial populations and optimal moisture conditions. Aged casts showed reduced enzyme activities because of a decreased moisture content, lower nutrient concentrations and a decline in microbial activity. Earthworm-microbe symbiosis seems to operate in a stepwise fashion, controlling groups of enzymes during the metabolism of PM to keep the flux of nutrients in casts (soil) in balance in the biosphere.

Yim and Tam (1999) studied the effects of waste water-borne heavy metals on growth of young plants (9-month-old *Bruguiera gymnorrhiza*) and soil microbial activities in mangrove microcosms. During the 26-week loading period, each mangrove microcosm received 31.2 litres synthetic waste water of three strengths: normal, medium (5 times of normal strength) and strong (10 times of normal strength). Normal strength wastewater had Cu, Zn, Cd, Cr and Ni concentrations of 3, 5, 0.2, 2 and 3 mg L^{-1}, respectively. Plant growth and total plant biomass in wastewater-treated microcosms were lower than that in the control, and the maximum reduction was found in microcosms receiving strong wastewater. Alkaline phosphatase activity and ATP contents of the mangrove soils receiving wastewater were also reduced. More than 95% reduction in these two parameters was found in soils loaded with strong wastewater. Microtox test demonstrated that soil elutriates obtained from

microcosms receiving strong waste water were of the greatest toxicity (EC50 was 23%), These results show that high concentrations of heavy metals present in strong wastewater were toxic and posed negative effects to both mangrove plants and soil microbial activities. Microbial activities were generally more sensitive to the toxicity of heavy metals than plants.

Speir *et al.* (1999) reported that New Zealand soils of contrasting texture, organic matter content and cation exchange capacity (CEC) were amended with solutions of the nitrate salts of Cd[II], Cr[III], Cu[II], Ni[II], Pb[II] and Zn[II], spanning the concentration range, 0-100 mmol heavy metal kg^{-1} soil. Additional treatment sets comprising: 1) the same range of $Ca(NO_3)_2$ concentrations to account for osmotic effects, and 2) the same range of NO^-_3 concentrations, comprising $NaNO_3$ acidified with HNO_3, to account for the acidifying effects of metal salt amendment of the soils, were also included. Samples were assayed for phosphatase and sulphatase enzyme activities and for basal respiration and substrate-induced respiration (SIR), approximately 1 week after amendment. Metal amendment resulted in considerable acidification of all three soils, with the metals which hydrolyse most (Cr, Cu and Pb) having the greatest effect, and the coarsest textured soil being the most affected. Phosphatase activity declined up to 70% as a result of metal amendment in the fine-textured soils, but acid amendment had little or no effect. In the coarse-textured soil, neither acid nor most metals inhibited phosphatase activity until pH fell to below 4. In contrast, sulphatase activity was strongly inhibited by acid and by all metal amendments including Ca, in all three soils, indicating that acidification was the dominant effect. In another experiment the relations between anthropogenic environmental pollution and the level of inorganic phosphorus in soil, enzyme activities of extracellular soil acid phosphatase and the surface acid phosphatase of excised ectomycorrhizas of Scots pine (*Pinus sylvestris* L.) were studied. Soil and root samples were taken from two Scots pine stands in central Poland: a polluted site exposed to long-term pollution from a steelworks and the city of Warsaw and a reference plot (control) free from direct impact of pollution. The polluted site was characterised by high concentration of trace elements (Cd, Pb, Cu, Zn, Mn, Cr) and low level of inorganic phosphate in soil. This site had significantly lower enzyme activities of soil acid phosphatase (0.54 mu moles p-nitrophenol released g^{-1} dry weight h^{-1}) and surface acid phosphatase of pine ectomycorrhizas (3.37 mu moles p-nitrophenol released g^{-1} fresh weight h^{-1}) than the control site (1.36 mu moles p-nitrophenol released g^{-1} dry weight h^{-1} and 12.46 mu moles p-nitrophenol released g^{-1} fresh weight h^{-1}, respectively). The levels of phosphate, carbon and nitrogen in pine fine roots were also analysed. Low concentrations of PO^-_4 P and high N: P ratio in pine fine roots from polluted site were found. The results suggest that soil pollutants may have a negative effect on the extracellular acid phosphatase of soil and Scots pine ectomycorrhizas and on the phosphorus status in fine roots of the plant (Kieliszewska-Rokicka, 1999).

Correlations: Senwo and Tabatabai (1999) found that the activity of aspartase enzyme in soils was significantly correlated with the activities of asparaginase, glutaminase, urease and amidase. The soil properties that related to the amounts of L-glutaminase activities in 25 surface soils included organic carbon and total nitrogen. There was no significant relationship between L-glutaminase activity and pH, percentage of clay or sand. There was, however, a significant correlation between L-glutaminase activity and amidase, urease and L-asparaginase activities in the surface samples studied (Frankenberger and Tabatabai, 1991a).

Bergstrom *et al.* (1998) measured the spatial dependence of soil enzyme activities and other properties of the Ap horizon in a Grey Brown Luvisol (Hapludalf). Soil samples were collected at 74 positions along a slope following harvest of soybean [*Glycine max* (L.) Merr.] and fall tillage. Parameters measured were activity of dehydrogenase, urease, glutaminase, phosphatase, arylsulfatase and beta-glucosidase; water, organic carbon (OC), mineral N, and inorganic P contents; the light fraction of soil organic matter and depth of the Ap horizon. Rank correlation indicated significant relationships between water and dehydrogenase, urease, glutaminase, phosphatase and arylsulfatase activities, and between water and OC content. Depth of the Ap horizon, water content and arylsulfatase activity were strongly spatially dependent; OC and inorganic P contents and phosphatase activity were moderately spatially dependent.

Fernandes *et al.* (1998) conducted a greenhouse study using a Dusky-Red Latosol (Oxisol) and a Structured Dusky-Red Earth (Ultisol) under three use conditions and four practices of correction of soil fertility with the objective of studying the forms of P in soil, the phosphatase activity and the P extracted by Mehlich 1, Mehlich 3 and resin. Bean plants were cultivated with the objective of correlating the dry matter production with the soil parameters studied. In the Oxisol, independently of use conditions and of practices of fertility correction, it was observed dominance of fraction of P linked to iron, while in the Ultisol higher amounts of P linked to aluminium and calcium were observed. The use conditions had great influence upon organic forms of P and phosphatase activity, being the soils under forest the ones that presented higher amounts of total organic P, organic P in microbial biomass and higher activity of acid and alkaline phosphatases. The three extractants presented positive and significative correlations with the forms of soil inorganic P and with the plant parameters evaluated.

Olander and Vitousek (2000) measured acid phosphatase and chitinase (N-acetyl ss-D-glucosaminide) activity in soil across a chronosequence in Hawaii where N and P availability varies substantially among sites and long term fertilizer plots had been maintained for over 4 years. Phosphatase activity was high at all sites. Chitinase activity decreased significantly as age and N availability increased across the chronosequence. Phosphorus addition suppressed phosphatase activity at all sites, while N addition increased phosphatase activity at the young, N-limited site. In contrast, N addition repressed chitinase activity only at the N limited young site, and P additions had no effect on chitinase activity. These results suggest that the regulatory relationship between nutrient supply and nutrient mineralization are asymmetric for N and P, and that the differences could help to explain differences observed in patterns of N and P availability.

Marinari *et al.* (2000) studied in a field experiment the influence of different fertilizer applications on biological and physical properties of soil. Vermicompost (VC) from biological sludge, stabilised dairy manure or mineral nitrogen fertilizer (NH_4NO_3) were applied to a corn crop (*Zea mays* L.) at 200 kg N ha^{-1}. Soil enzyme activity (acid phosphatase, dehydrogenase and protease) and CO_2 production were measured as indices of soil biological activity. These measures of metabolic activity were correlated to physical properties such as porosity of soil. The soluble fractions of C and N were taken as indicators of fertilizer effects on soil fertility. There were positive correlations between soil porosity, enzymatic activity and CO_2 production in organic and mineral treatments. The addition of organic fertilizers improved soil physical and biological properties. The increase in macropores in soil treated with organic fertilizers was mainly due to an increase in elongated pores, which are considered very important both in soil-water-plant relationships and in maintaining a good soil structure. Organic treatments stimulated soil biological activity probably due to an enrichment of soil organic matter. Mineral fertilizer enhanced soil porosity by increasing regular and irregular pores

and caused a priming effect of native soil organic matter.
Sarapatka and Krskova (1997) revealed that soil phosphatases play a major role in the mineralization processes of organic phosphorus substrates. Their activity can be influenced by numerous factors and soil properties play a key role among them. This research adds to the growing knowledge on soil phosphatases and their interactions with the specific soil characteristics of nine sites in the Czech Republic with common soil types. The results show correlations and linear equations between phosphatase activity and some soil characteristics. Positive correlations were found between enzymatic activity and organic carbon, and with nitrogen, and between acid phosphatase activity and total phosphorus. Negative correlations were with the quality of humus (humic : fulvic acids ratio) and available phosphorus, and between acid phosphatase activity and clay content and pH.
Pagliai and Denobili (1993) studied porosity and pore size distribution from thin sections, prepared from undisturbed Ap horizon samples taken from a zero-versus conventional-tillage field experiment. The samples were analysed by means of a Quantimet 720 image analysing computer. The length and the size distribution of plant roots were determined using the same image analyzer. Soil urease and phosphatase activity in soil samples from the plots of this field experiment was also determined. Total porosity was significantly higher in conventionally tilled plots, but the proportion of pores ranging from 30 to 500 mm in equivalent pore diameter, which are considered the most important both in soil-water-plant relationships and in the maintenance of a good soil structure, was higher in no-tilled plots. Root development showed a strict relationship with the presence of smaller pores which were more numerous in no-tilled plots. Enzyme activity was also higher in these plots than in the conventionally tilled plots. The relationships of such enzyme activities and the various pore size classes in each type of soil showed a positive common trend between the two enzyme activities and the percentage area of pores ranging from 30 to 200 mm in equivalent pore diameter. A significant correlation was observed between the degree of porosity in this range and urease activity.

Applications of enzymes in soil-plant environment: Dick and Tabatabai (1993) while reviewing the significance and potential uses of soil enzymes have enumerated various applications of enzymes in soil–plant environment. Some of these are discussed as under:

Enzyme activities as an index of soil fertility: Hofmann and Seegerer (1950) proposed the activity of enzymes as an index of the fertility status of the soil. Enzymes essentially integrate the effects of climate, cultivation, soil amendments, and edaphic properties, resulting in an activity indicative of the soil's fertility (Skujins, 1978). He, however, concluded that obtaining a "fertility index" by the use of any soil enzymatic activity seemed unlikely. Soil enzyme activities are substrate specific, closely related to organic matter levels, and are unable to reflect the total nutrient status of the soil. In addition, even sterile sands can be considered "fertile," provided they include the right mix of nutrients and water.
Economic and environmental consequences that result when the fertility status of the soil (especially nitrogen fertility) is misdiagnosed continue to stimulate research into development of new ways of measuring soil fertility. For farming systems in which soil fertility strongly tied to the turnover of organic matter (i.e., organic farming and sustainable agriculture systems), a closer relation may exist between enzyme activities and a soil's fertility.
The integrative activity of several soil enzymes has recently been proposed as a means to predict a soil's fertility. This approach might better reflect both the release of nutrients during organic matter turnover and the relative availability of inorganic nutrients compared with the activity of any single enzyme. Various enzymes release specific plant nutrients from soil organic matter, and their activity correlates with the fertility status of the soil, as related to the availability of that nutrient. For example, several carbohydrates are involved in litter decomposition by the fungus *Lycopodiumtristachyum*, which yields fairy rings in lawns, with the net result being an increase in available P and N (Spalding and Duxbury, 1977).
Positive relations were observed between acid phosphatase and various forms of soil P (Baligar *et al.*, 1988), and between acid phosphatase and alkaline phosphatase activity and the depletion of organic P in the rhizosphere of wheat ($r = 0.99$) and clover ($r = 0.97$) (Tarafdar and Jungk, 1987). Acid phosphatase was also significantly and positively correlated with wheat yields (Dick *et al.*, 1988). Speir (1984) suggested that soil sulfatase may be used an index for S nutrition in soils from Tonga. Significant positive correlations were observed between sulfatase activity and absorbed S and yields of green panic (*Panicum maximum*) with all nutrients supplied except S. Speir (1977) also observed a strong correlation between sulfatase activities and amounts of available S in New Zealand soils.
Dalton and colleagues (1985) reported a novel approach that shows promise for evaluating the availability of micronutrients in soils. Urease, which has a Ni requirement, was used to assess the availability of that metal. Soils were treated with Ni and the changes in urease activity were observed. The greater the stimulation of urease activity caused by Ni additions, the lower the original level of available Ni in the soil. No stimulation reflected an adequate level. Presumably, this approach could be used to assess the availability of other trace elements, provided a suitable enzyme requiring that trace element can be assayed in soil.

Fertilizer use efficiency: Soil enzymes produce compounds that plants can utilize from several fertilizers. The most commonly used fertilizer of which this is true is urea. Other fertilizer compounds proposed as sources of plant nutrients, such as substituted amides or condensed phosphatase also require soil enzymatic activity to release the nutrient in a plant-available form.
Soil urease hydrolyzes urea to form ammonium carbonate resulting in increased pH and ammonia volatilization- a nitrogen loss. Several approaches have been investigated to reduce ammonia losses from urea. These include 1) soil incorporation or deep placement, 2) use of slow-release forms of urea fertilizer, and 3) use of soil urease inhibitors.
Numerous compounds have been tested to determine their effectiveness as urease inhibitors. For urease inhibitors to be an effective technology, however, they must meet several criteria. They must be inexpensive, specific in inhibition of urease at low concentration, easily applied, compatible with urea, easy to store, biodegradable at appropriate rates, environmentally innocuous, and able to move through the soil with urea fertilizer.
The fertilizers composed of compounds with high N or P contents, also require the activity of enzymes in soils before the nutrients become available. These compounds include low molecular weight amides, purine and pyrimidine bases, and condensed phospates; the value of pyrophosphate as a fertilizer source of P is dependent on its rate of hydrolysis (Sutton *et al.*, 1966). Many reactions involving fertilizer compounds are simple hydrolytic reactions, and several soil enzymes involved have been characterized.

Biologically active substances: Biologically active soil compounds, naturally occurring organic compounds which influence plant growth at extremely low concentrations are also termed as plant growth regulators (Arshad and

Frankenberger, 1998). The use of plant growth regulators for increasing crop yields is gaining attention. Libbert and Paetow (1962) first demonstrated that indole-3- acetonitrile and indol-3- acetaldehyde were hydrolyzed in soil to form indole-3-acetic acid (IAA) and indole-3-carboxylic acid, respectively. Sarwar *et al.* (1992) reported formation of IAA from tryptophan in soil, and a soil extract was found to have similar activity (Chilvignac and Mayaudon, 1971). Auxin production was increased in pure culture when rhizobacteria were supplemented with L-tryptophan (Zahir *et al.*, 2000, Asghar *et al.*, 2000).

Toluene was included in these assay procedures suggesting that the enzyme involved in the production of IAA was no longer associated with living cells. Similar result were obtained for the plant hormone ethylene when Lynch (1974) reported that a filtered, extracellular preparation of *Mucor hiemalis* produced 60- fold more ethylene than the same culture growing in sealed flask. Primrose (1976) measured similar amounts of ethylene evolved from cell suspensions and cell-free filtrates of *Escherichia coli*. Frankenberger and Phelan (1985a,b) studied ethylene production in soil from 1-aminocyclopropane-1-carboxylic acid (ACC), an immediate precursor in the biosynthetic pathway for ethylene. Significant amounts of ethylene were produced from air-dried soils within 24 hr and without a lag period, further suggesting the participation of extracellular soil enzymes.

Increased levels of enzymes often occur in the rhizosphere, and their presence lends specific advantages to the plant (Curl and Truelove, 1986; Rovira and Mc-Dougall, 1967). Sources of activity are intact plant cells on the root surfaces, sloughed off root cells, enzymes secreted by plant or microbes, or active rhizosphere microbes (Dodd *et al.*, 1987). The exploitation of enzymes in the plant root rhizosphere, whether or not extracelular, that can rapidly convert precursors to biologically active molecules, holds potential for increasing crop production. Low concentrations of L-tryptophan, for example, stimulated both shoot and root growth of Douglas fur (*Pseudotsuga menziesii*) seedling inoculated with the fungus *Pisolithustinctorius* (Frankenberger and Poth, 1987). Enhanced production of cytokinins by rhizosphere microorganisms, with adenine and isopentyl alcohol added as synthetic precursors, has also been observed (Nieto and Frankenberger, 1989).

Amylase, cellulase, proteases, lipases, phosphatase, and sulfatases can also act upon organic substrates in the rhizosphere to make mineral elements available (Burns, 1978; Dodd *et al.*, 1987; Estermann and McLaren, 1961). Acid and alkaline phosphatase activities in wheat rhizosphere were strongly correlated with the depletion of organic P (Trafdar and Jungk, 1987).

The harnessing of enzyme activities in the rhizosphere to provide a means to directly affect plant growth now seems possible. What is needed is a substrate easily convertible to an essential plant nutrient or to a biologically active compound by one or only a few simple steps. Several benefits of this approach are possible. For biologically active compounds, it may be less costly to apply a synthetic precursor than the compound itself. Second, the compounds applied are relatively benign until acted upon, offering a measure of safety.

The plant roots may also be possible to engineer to secrete large amounts of a given enzyme, such as a specific phosphatase. If fertilized with the proper organic substrate, only plants capable of releasing the appropriate phosphatase would utilize the added P. Similarly plants could be engineered to secrete enzymes into the rhizosphere to degrade or detoxify pollutants.

Oxidation-reduction status of soil: The influence of flooding and reduced O_2 on the activity of several enzymes has also been investigated by several workers. Waterlogging and reduction caused a 2.5 to 6 fold increase in rhodanese activity in a pokkali (acid sulfate) soil; but no increases occurred when an alluvial soil was flooded (Ray *et al.*, 1985). A positive correlation between O_2 diffusion rate and catalase activity was reported by Glinski and co-workers (1986). After a soil was maintained for 7 days in a waterlogged condition, acid phophatase, alkaline phosphatase, urease, and arylsulfatase activities were positively correlated with redox potential, whereas phsphodiesterase and amidase were negatively correlated (Pulford and Tabatabai, 1988).

Indication of pollution: The most valuable single use of soil enzymes is to assess the effects of various inputs on the relative health of the soil. Numerous studies have been conducted to determine changes in a soil's enzyme activities caused by acid rain, heavy metals, pesticides, and other industrial and agricultural chemicals.

There are differences between how heavy metals and xenobiotic agents affect soil enzyme activities. Generally, concentrations of heavy metals (especially Hg, Ag, Cd and Cu) required to bring about a biological effect are lower than for xenobiotics. A technology has been developed whereby trace amounts of Cu are added to histosols to prevent carbohydrase activity and, thus, the mineralization and loss of organic matter from these very productive soils (Mathur *et al.*, 1980; Mathur and Sanderson, 1980). As enzyme components, many metals are also required at low concentrations for optimum biological activity. For example, Ni is an essential part of urease. Tena and co-workers(1981), and Dick and Tabatabai (1983) also found that Mg, Ca, Ba, Co, Ni, Zn and Mn activated soil pyrophosphatase activity, presumably by formation of a substrate-metal-enzyme bridge.

Many studies have involved the use of pesticides, the addition of which can either stimulate or inhibit soil enzyme activities (Ladd, 1985). If recommended field application rates are used, inhibitory results are temporary, and enzyme activities return to levels similar to those in untreated soils in a few weeks or months. Zantua and Bremner(1977) have proposed that soils contain a baseline or background level of enzyme activity that is very difficult to permanently change.

Remediation of contaminated soil: Sufficient evidences in the literature for the involvement of soil enzymes in the degradation of organic compounds are scattered throughout the literature. However, the number of studies investigating this possibility are less than one might expect. Research has been conducted in which the native levels of soil enzyme activities are used to bring about compound transformation. An increase in the rates of reactions can be achieved by amending the soil to increase its overall biological activity, in the hope that enzymes involved in transformation of the organic compounds will increase in direct proportion. An inherent lack of specificity is a major limitation of this approach, although addition of organic amendments would also serve to remove or stabilize the pollutant by its incorporation into soil organic matter.

Repeat applications of a single pesticide, or chemically similar pesticide, may lead to the pesticide being so rapidly degraded that it is rendered ineffective almost immediately after its application. A chemical example as how soil enzymes are involved in this phenomenon, called enhanced biodegradation, has been reported for the insecticides insofenfos and fonofos (Murphy and Minor, 1986; Sikora and Kaufman, 1987). After repeat applications of the insecticides to soil, the activity of a phosphatase, which ctalysed the hydrolytic removal of the phosphate group, occurred at a greatly increased rate. Enhanced biodegradation has been reviewed by Racke and Coats (1990).

The involvement of soil enzymes in the degradation of other organophosphorus insecticides and acylanilide herbicides has

been reviewed by Burns and Edwards (1980). In particular, the organophosphorous insecticides were found to degrade faster in irradiated soils than in those that had been autoclaved (Burns and Gibson, 1980; Getzin and Rosefield, 1968).

Additional evidence for soil enzyme involvement in the transformation of organic compounds comes from studies in which soils are first extracted with a buffer or other suitable extractant, and the extract is then used as a crude source of enzyme activity. Peroxidase extracts from several soils converted chloroanilines to chlorobenzenes (i.e. degradation products of phenylamide herbicide) (Bartha and Bordeleau, 1969). Getzin and Rosefield (1968, 1971) extracted from soil a cell-free enzyme able to degrade malathion. An enzymatic system, tentatively identified as a lacasse of microbial origin, was extracted from soil with neutral sodium pyrophosphate (Ruggiero and Radogna, 1984). Bollag *et al.* (1987) studied the extraction and purification of peroxidase from soil.

Transformation of organic compound in soil by soil enzymes can involve several different type of processes. Filip and Preusse (1985) reviewed the chemical nature of phenoloxidases and concluded that these enzymes can protect soils against the accumulation of harmful organic compounds by catalysing reactions involving degradation, polymerization, synthesis, coupling, and incorporation into humic complexes. Enzymatic coupling of certain organic compounds is an important natural process that affects their fate (i.e. reactivity and persistence) in soil and water. (Sarkar *et al.*, 1988; Sjoblad and Bollag, 1981).

Direct additions to soil of purified or partially purified enzymes to increase transformation rates of organic compounds in soil have been investigated. Generally, high rates are required , and the effect is only temporary (Dick and Tabatabai, 1983, Shannon and Bartha, 1988). Heuer and co-authors (1976) reported that adding phosphatase to soil extract containing an organophosphorous insecticide increased the rate at which the insceticide was degraded. Addition of the same enzyme to soil, however, yielded no effect. Peroxidase or laccase effectively immobilized phenolic compounds in a sandy soil, but the high rates of enzyme addition (50-425 units of activity) and the use of sandy soil make extension of this technique to other soil systems impractical (Shannon and Bartha, 1988). Work by Dick and Tabatabai (1983) and Hope and Burns (1987) demonstrated that free enzymes in a partially purified form are immediately and effectively inhibited when added to soil. This was attributed to masking of the active sites when enzymes became bound to solid particles in the soil and to inhibition by soluble salts, together with biological degradation of the enzyme.

Specific enzymes may be delivered to soil after first being immobilized onto solid supports to avoid the rapid loss of activity when enzymes are added directly to soil. Usually, immobilization decreases reaction rates, but significantly more activity is retained than if the same amount of free enzyme had been added directly to soil. Immobilization also imparts a certain amount of stability against temperature extremes, proteolysis and the presence of inhibitory factors. Addition of soluble laccase and glucose oxidase to soil suspensions resulted in 100 and 85% loss of activity, respectively, in 15 days, whereas these same enzymes immobilized onto clays lost only 12 and 25% of their activity over the same 15-day period (Sarkar *et al.*, 1989). An inherent weakness of immobilized enzymes, however, is their inability to act on water-insoluble substrates. The substrate must diffuse to the immoblized enzyme for a reaction to occur.

Numerous studies have been reported in which enzymes have been immobilized in to soil components, such as clays, humus, or a mixture of the two (Hartmeier, 1988; Mosbach, 1987, 1988; Rosevear *et al.*, 1987; Woodward, 1985). Advatages of using immobilized enzymes for transformation of organic compounds in soil are that 1) it is not necessary to release genetically engineered microorganisms, 2) immoblized enzymes may be reused and are more tolerant of toxic concentrations of contaminants, and 3) competition with existing soil microflora is avoided. For this technology to be practical, however, a readily available source of enzymes is required. This can be obtained by growing large numbers of cells and harvesting the cells for enzyme content. However, if enzyme yields from growing cells are low, it may be possible to insert the gene(s) responsible for the desired enzyme into a more appropriate organism capable of effectively directing both the synthesis and secretion of enzymes into the growth medium. A large amount of genetic material with potential for effectively transforming organic compounds could thus be utilized. One potential source of degradative genes are microorganisms known to be responsible for the enhanced biodegradation of pesticides in soil.

Conclusions: Enzymes-the biomloecules dependent on pH, ionic strength, temperature and presence or absence of inhibitors; are essential for the solublization and degradation of chemicals (fertilizers and pesticides etc.) which help a lot in the continuation of the ecosystem in the soil and the environment as well. These have significant and potenial applications in soil-plant environment such as an index of soil fertility, indication of pollution, oxidation-reduction status of soil and remediation of contaminated soil.

References

Arshad, M. and W.T. Frankenberger, Jr., 1998. Plant growth regulating substances in the rhizosphere: Microbial Production and Function. Advances in Agron., 62: 45-151.

Asghar, H.N., Z.A. Zahir, A. Khaliq and M. Arshad, 2000. Isolation of different rhizobacteria from different rapeseed varieties and their potential for auxin production. Pak. J. Bio. Sci., 3: 1556-1559.

Baligar, V.C., R.J.Wright and M.D. Smedley, 1988. Acid phosphatase activity in soils of the Appalachian region. Soil Sci. Soc. Am. J., 52: 1612-1616.

Bartha, R. and L. Bordeleau, 1969. Cell-free peroxidases in soil. Soil Biol. Biochem., 1:139-143.

Bergstrom, D.W. , C.M. Monreal, J.A. Millette and D.J. King,1998. Spatial dependence of soil enzyme activities along a slope. Soil Sci. Soc. Am. J., 62: 1302-1308.

Bollag, J.M., C.M. Che, J.M. Sarkar and M.J. Loll, 1987. Extraction and purification of a peroxidase from soil. Soil Biol. Biochem., 19:61-67.

Bonmati, M., B. Ceccanti and P. Nanniperi, 1991. Spatial variability of phosphatase, urease, protease, organic carbon and total nitrogen in soil. Soil Bio. Biochem., 23: 391-396.

Bopaiah, B. M., 1990. Microbiological and enzyme activities profile in the root zone and interspace soils of coconut and arecanut palms. J. Plantation Crops, 18: 50-54.

Browman, M. G. and M. A. Tabatabai, 1978. Phosphodiesterase activity in soils. Soil Sci. Soc. Am. J., 42: 284-290.

Burns, R. G., 1978. Enzyme activity in soil: Some theoretical and practical considerations. In: Burns, R.G. (Ed.), Soil Enzymes, Academic Press , New York, pp: 295-340.

Burns, R.G. and J.A. Edwards,1980. Pesticide breakdown by soil enzymes. Pestic. Sci., 2: 506-512.

Burns, R.G. and W.P. Gibson, 1980. The disappearance of 2,4-D, diallate and malathion from soil and soil components. In: Banin, A., Kafkafi, U. (Eds.), Agrochemicals in Soil. Pergamon Press, Oxford, pp: 149-159.

Cashel, M. and E. Freese, 1964. Excretion of alkaline phosphatase by *Bacillus subtilis*. *Biochem. Biophys. Res. Commun.*, 16: 541-544.

Chalvignac, M.A. and J. Mayaudon, 1971. Extraction and study of soil enzymes metabolizing tryptophan. Plant Soil, 34: 25-31.

Cochran, V. L., L.F. Elliott and C. E. Lewis, 1989. Soil microbial biomass and enzyme activity in subarctic agricultural and forest soils. Biol. Fertility Soils, 7: 283-288.

Curl, E.A. and B. Truelove, 1986. The rhizosphere. Springer-Verlag, New York.

Dalton, D.A., H.J. Evans and F.J. Hanus, 1985. Stimulation by nickel of soil microbial urease activity and urease and hydrogenase activities in soybeans grown in a low-nickel soil. Plant Soil, 88:

Dick, R.P., P.E. Rasmussen and E.A. Kerle, 1988. Influence of long-

term residue management on soil enzyme activities in relation to soil chemical properties of a wheat fallow system. Biol. Fertil. Soils, 6: 159-164.

Dick, W. A., and M. A. Tabatabai, 1978. Inorganic pyrophosphatase activity of soils. Soil Biol. Biochem., 10: 59-65.

Dick, W.A. and M.A. Tabatabi, 1983. Activation of soil phosphatase by metal ions. Soil Biol. Biochem., 15: 59-363.

Dick, W.A. and M. A. Tabatabai, 1993. Significance and potential uses of soil enzymes. In: Metting, F.B. Jr. (Ed.), Soil Microbial Ecology. Marcel Decker, Inc., New York, USA.

Dodd, J.C., C.C. Burton, R.G. Burns and P. Jeffries, 1987. Phosphatase activity associated with the roots and the rhizosphere of plants infected with the vesicular-arbuscular mycorrhizal fungi. New Phytol., 107: 163-172.

Eivazi, F. and M. A. Tabatabai, 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem., 20: 601-606.

Estermann, E. F. and A. D. McLaren, 1961. Contribution of rhizosplane organisms to the total capacity of plants to utilize organic nutrients. Plant Soil, 15:243-260.

Fernandes, L.A., A.E.F. Neto, N. Curi, J.M. De Lima and G.A.A. Guedes, 1998. Phosphorus and acid phosphatase activity in two soils under different use conditions. Pesquisa Agropecuaria Brasileira, 33: 1159-1170

Filip, Z. and T. Preusse, 1985. Phenoloxidases-their properties and effects in soil. Pedobiologia, 38: 133-142.

Frankenberger, Jr., W. T. and M. A. Tabatabai, 1991a. Factors affecting L-Glutaminase activity in soils. Soil Biol. Biochem., 23: 875-879.

Frankenberger, Jr., W. T. and M. A. Tabatabai, 1991b. L-Glutaminase activity of soils. Soil Biol. Biochem., 23: 869-874.

Frankenberger, Jr., W.T. and M. Poth, 1987. Biosynthesis of indole-3-acetic acid by the pine ectomycorrhizal fungus *Pisolithus tinctorious*. Appl. Environ. Microbiol., 53: 2908-2913.

Frankenberger, Jr., W.T. and P.J. Phelan, 1985 a. Ethylene biosynthesis in soil: I. Method of assay in conversion of 1 aminocyclopropane-1-carboxylic acid to ethylene. Soil Sci. Soc. Am. J., 49: 1416-1422.

Frankenberger, Jr., W.T. and P.J. Phelan, 1985 b. Ethylene biosynthesis in soil: II. Kinetics and thermodynamics in the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. Soil Sci. Soc. Am. J., 49: 1422-1426.

Garcia, C., T. Hernande, F. Costa, B. Ceccanti and C. Ciardi, 1992. Changes in A+P content, enzyme activity and inorganic nitrogen species during composting of organic wastes. Can. J. Soil Sci., 72: 243-253.

Getzin, L.W. and I. Rosefield, 1968. Organophosphorous insecticide degradation by heat-labile substances in soil. J. Agric. Food Chem., 16: 598-601.

Getzin, L.W. and I. Rosefield, 1971. Partial purification and properties of a soil enzyme that degrades the insecticide malathion. Biochem. Biophys. Acta, 235: 442-453.

Glinski, J., Z. Stepniewska and M. Brezezinska, 1986. Characterization of the dehydrogenase and catalase activity of the soils of two natural sites with respect to the soil oxygenation status. Pol. J. Soil Sci., 19: 47-52.

Grejtovsky, A., 1991. Effect of improvement practices on enzymatic activities of heavy-textured alluvial soil. Posttinna-Vyroba, 37: 289-295.

Guan, S. Y., 1989. Studies on the factors influencing soil enzyme activities: I. Effects of organic manures on soil enzyme activities and N and P transformations. Acta-Pedologica-Sinica, 26: 72-78.

Gupta, R.D. and K.K.R. Bhardwaj, 1990. Phosphatase and urease enzymic activities in some soil profiles of North West Himalayas. J. Ind. Soc. Soil Sci., 38: 756-759.

Hartmeier, W., 1988. Immobilized Bioctalysts: An Introduction. Springer-Verlag, New York.

Heuer, B., I. Birk and B. Yaron, 1976. Effect of phosphatases on the persistence of organophosphorous insecticides in soil and water. J. Agric. Food Chem., 24:611-614.

Hofmann, E. and A. Seegrer, 1950. Soil enzymes as a measure of biological activiy. Biochem. Z., 321:97.

Hope, C.F.A. and R.G. Burns, 1987. Activity, origin and location of cellulases in a silt loam soil. Biol. Fertil. Soils, 5:164-170.

Hysek, J. and B. Sarapatka, 1998. Relationship between phosphatase active bacteria and phosphatase activities in forest soils. Biol. Fertil. Soils, 26:112-115.

Iftikhar, A. and K. M. Khan, 1988. Studies on enzymes activity in normal and saline soils. Pak. J. Agri. Res., 9:505-508.

Juma, N. G., 1976. Phosphatase activity of soils and of corn and soybean roots. M. S. Thesis. Iowa State Univ., USA.

Juma, N. G. and M. A. Tabatabai, 1988. Phosphatase activity in corn and soybean roots: Conditions for assay and effects of metals. Plant Soil, 107: 39-47.

Khan, M. R. 1989. Basic Enzymology. In Biochemistry vol. I pp: 257-327.

Kieliszewska-Rokicka, B., 1999. Phosphate status and acid phosphatase activity in soil and ectomycorrhizas in two mature stands of Scots pine (Pinus sylvestris L.) exposed to different levels of anthropogenic pollution. Acta Societatis Botanicorum Poloniae, 68: 311-317.

Kim, J. E. and J. U. Hong, 1988. Effects of herbicides on enzyme activities in soil environment. J. Korean Agri. Chem. Soc., 31:79-85.

Kramer, S and D.M. Green, 2000. Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semiarid woodland. Soil Biol. Biochem., 32: 179-188.

Ladd, J.N., 1978. Origin and range of enzymes in soil. In: Burns, R.G. (Ed.), Soil Enzymes. Marcel Dekker, New York, pp: 51-96.

Ladd, J.N., 1985. Soil enzymes. In: Vaughan, D., Malcolm, R.E. (Eds.), Soil Organic Matter and Biological Activity. Martinus Nijhoff, Boston.

Libbert, E. and W. Paetow, 1962. Investigations of the enzymatic hydrolysis of indole-3-acetonitrile and of the oxidation of indole-3-aldehyde in the soil and in the unboiled milk. Flora Jena, 152: 540-544.

Lynch, J.M., 1974. Mode of ethylene formation by *Mucor hiemalis*. J. Gen. Microbiol., 83: 407-411.

Marinari, S., G. Masciandaro, B. Ceccanti and S. Grego, 2000. Influence of organic and mineral fertilizers on soil biological and physical properties. Bio Resource Tech., 72: 9-17.

Mathur, S.P. and R.B. Sanderson, 1980. The partial inactivation of degradative soil enzymes by residual fertilizer copper in histosols. Soil Sci. Soc. Am. J., 44: 750-755.

Mathur, S.P., J.I. MacDougall and M. McGrath, 1980. Levels of activities of some carbohydrases, protease, lipase, and phosphatase in organic soils of differing copper content. Soil Sci., 129: 376-385.

Mosbach, K., 1987. Immobilized enzymes and cells. Methods Enzymol., Part B, 135: 3-172.

Mosbach, K., 1988. Immobilized enzymes and cells. Methods Enzymol., Part D, 137: 583-712.

Murphy, J.J. and R.G. Minor. 1986. Distribution of a soil microbe involved in the enhanced degradation of the insecticide Amaze. Agron. Abst., p:185.

Neitzke, M., 1999. Microbiological properties along transects in calcareous grasslands. J. Plant Nutr. Soil Sci. Zeitschrift Fur Pflanzenernahrung und Bodenkunde, 162: 637-644.

Nieto, K.F. and W.T. Frankenberger Jr., 1989. Biosynthesis of cytokinins by *Azotobacter chroococcum*. Soil Biol. Biochem., 21: 967-972.

Olander, L.P. and P.M. Vitousek, 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry, 49: 175-190.

Pagliai, M and M. Denobili, 1993. Relationships between soil porosity, root development and soil enzyme-activity in cultivated soils. Geoderma, 56: 243-256.

Parthasarathi, K. and L.S. Ranganathan, 2000. Aging effect on enzyme activities in pressmud vermicasts of *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg). Biol. Fert. Soils, 30: 347-350

Perucci, P., 1990. Effect of the addition of municipal soild- waste compost on microbial biomass and enzyme activities in soil. Biol. Fert. Soils, 10: 221-226.

Primrose, S.B., 1976. Formation of ethylene by *Escherichia coli*. J. Gen. Microbiol., 95: 159-165.

Pulford, I.D. and M.A. Tabatabai, 1988. Effect of waterlogging on enzyme activities in soils. Soil Biol. Biochem., 20: 215-219.

Quastel, J. H., 1946. Soil Metabolism. The Royal Institute of

Chemistry of Great Britain and Ireland, London. Rostlinna-Vyroba., 37: 289-295.

Racke, K. and J. Coats, 1990. Advances in Enhanced Soil microbial Pesticide Degradation. Am. Chem. Soc., Washington, D.C.

Ramirez-Martinez, J. R. and A. D. McLaren, 1966. Some factors influencing the determination of phosphatase activity in native soils and in soils sterilized by irradiation. Enzymologia, 31: 23-28.

Ray, R.C., N. Behe rea and N. Sethunathan, 1985. Rhodanese activity of flooded and nonflooded soils. Soil Biol. Biochem., 17: 159-162.

Rosevear, A., J.F. Kennedy and J. Cabral. 1987., Immobilized Enzymes and Cells. Adam Hilgar, Bristol.

Rovira, A.D. and B.M. McDougall, 1967. Microbial and biochemical aspects of the rhizosphere. In: McLaren A.D. and Peterson G.H. (Eds.) Soil Biochemistry, Vol. I. Marcel Dekker, New York.

Ruggiero, P. and V.M. Radogna, 1984. Properties of laccase in humus-enzyme complexes. Soil Science, 138: 74-87.

Saa, A. , M.C. Trasar-Cepeda and T. Carballas, 1998. Soil P status and phosphomonoesterase activity of recently burnt and unburnt soil following laboratory incubation. Soil Biol. Biochem., 30: 419-428.

Salam, A. K., A. Katayama and M. Kimura, 1998. Activities of some soil enzymes in different land use systems after deforestation in hilly areas of West Lampung, South Sumatra, Indonesia. Soil Sci. Plant Nutr., 44: 93-103.

Salam, A.K. , E . Sutanto., Y. Desvia, A. Niswati, Dermiyati and M. Kimura, 1999. Activities of soil enzymes in fields continuously cultivated with cassava, sugarcane, and pineapple in middle terrace areas of Lampung Province, South Sumatra, Indonesia. Soil Sci. Plant Nutr., 45: 803-809.

Sarapatka, B. and M. Krskova, 1997. Interactions between phosphatase activity and soil characteristics from some locations in the Czech Republic. Rostlinna Vyroba., 43:415-419.

Sarkar, J.M. R.L. Malcolm and J.M. Bollag, 1988. Enzymatic coupling of 2,4-dichlorophenol to stream fulvic acid in the presence of oxidoreductases. Soil Sci. Soc. Am. J., 52: 688-694.

Sarwar, M., M. Arshad, D. A. Martens and W. T. Frankenberger, Jr., 1992. Tryptophan dependent biosynthesis of auxins in soil. Plant and Soil, 147: 207-215.

Senwo, Z. N. and M. A. Tabatabai, 1996. Aspartase activity of soils. Soil Sci. Soc. Am. J., 60: 1416-1422.

Senwo, Z. N. and M. A. Tabatabai, 1999. Aspartase activity in soils: effects of trace elements and relationships to other amidohydrolases. Soil Biol. Biochem., 31: 213-219.

Shannon, M.J.R. and R. Bartha, 1988. Immobilization of leachable toxic soil pollutants by using oxidative enzymes. Appl. Environ. Microbiol., 54: 1719-1723.

Shendzhen, A. Kh., E. P. Aleshin and O.A. doseeva, 1991. Change in the fermentative activity of rice field soil under the influence of microfertilizers. Doklady-Vsesoyuznoi-Ordena- Lenina-I-Ordena-Trudovogo- Krasnogo- Znameni- Akademii- Sel Skokhozy aistvennykh- Nauk- in- V. I.- Lenina, 8: 16-18.

Shukla, A.K. B. K. Tiwari and R. R. Mishra, 1989. Temporal and depthwise distribution of microorganisms, enzymes activities and soil respiration in potato field soil under different agricultural systems in north-eastern hill region of India. Reve-d' Ecolodie-et-de-Biologie-du-sol., 26: 249-265.

Sikora, L. J. and D. D. Kaufman, 1987. Enzyme activity in soil with accelerated pesticide degradation history. Agron. Abst., p: 192.

Sjoblad, R.D. and J.M. Bollag, 1981. Oxidative coupling of aromatic compounds by enzymes from soil microorganisms. In: Paul E.A. and. Ladd J.N. (Eds.). Soil Biochemistry. Vol. 5. Marcel Dekker, New York.

Skujins, J., 1978. History of abiontic soil enzyme research.. *In* R. G. Burns (Ed.) Soil Enzymes. Academic Press, New York, p:1-49.

Skujins, J. J., 1967. Enzymes in soil. Soil Biochemistry, Vol. 1, A. D. McLaren and G. H. Peterson (eds.). Marcel Dekker, New York, pp. 371-414.

Spalding, B.P. and J.M. Duxbury, 1977. Enzymatic activities and extrac table organic matter in soil invaded by *Lycopodium tristachyum*. Fairy Rings. Soil Sci. Am. J., 41: 1109-1113.

Speir, T. W., H.A. Kettles, H.J. Percival, A. Parshotam, 1999. Is soil acidification the cause of biochemical responses when soils are amended with heavy metal salts? Soil Biol. Biochem., 31: 1953-1961.

Speir, T.W., 1977. Studies on a climosequence of soils in tussock grasslands. 11. Urease, phosphatase, and sulphatase activities of top soils and their relationship with other properties including plant available sulphur. N.Z. J. Sci., 20: 159-166.

Speir, T.W., 1984. Urease, phosphatase, and sulphatase activities of Cook Island and Tongan soils. N.Z. J. Sci., 27: 73-79.

Spier, T. W., and D. J. Ross, 1978. Soil phosphatase and sulphatase. In: Burns R. G. (Ed.), Soil Enzymes. Academic Press, New York, pp: 197-250.

Staddon, W. J., L. C. Duchesne and J. T. Trevors, 1998. Acid phosphatase, alkaline phosphatase and arylsulfatase activities in soils from a jack pine (*Pinus banksiana* Lamb.) ecosystem after clear cutting prescribed burning, and scarification. Biol. Fertil. soils., 27: 1-4 .

Sutton, C.D., D. Gunary and S. Larsen, 1966. Phosphatase as a source of phosphorous for plants: II. Hydrolysis and initial uptake by a barley crop. Soil Sci., 101: 199-204.

Tabatabai, M. A., 1982. Soil enzymes. In: Page A. L., Miller R. H., Keeney D. R. (Eds.), *Methods of Soil Analysis*, Part 2, 2nd ed., Agronomy 9, American Society of Agronomy, Madison, Wis., pp: 903-947.

Tabatabai, M. A., 1994. Soil enzymes. In: R. W. *et al.* (Ed.), *Methods of Soil Analysis*, Part 2, Soil Science Society of America, Madison, pp: 797-798.

Tarafdar, J.C. and A. Jungk, 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorous. Soil Biol. Fertil., 3: 199-204.

Tena, M., J.A. Pinilla and R. Prado, 1981. Properties of inorganic pyrophosphatase in soil. An. Edafol. Agrobiol., 40: 1815-1824.

Tiwari, S. C., B. K. Tiwari and R. R. Mishra, 1989. Microbial community, enzyme activity and CO_2 evolution in pineapple orchard soil. Tropical Ecology, 30: 265-273.

Weimberg, R. and W. L. Orton, 1963. Repressible acid phosphomonoesterase and constitutive pyrophosphatase of *Saccharomyces mellis*. *J. Bacteriol.*, 86: 805-813.

Weimberg, R. and W. L. Orton, 1964. Evidence for an exocellular site for the acid phosphatase of *Saccharomyces mellis*. *J. Bacteriol.*, 88: 1743-1754.

Wick, B. , R.F. Kuhne and P.L.G. Vlek, 1998. Soil microbiological parameters as indicators of soil quality under improved fallow management systems in south-western Nigeria. Plant Soil, 202: 97-107

Woodward, J., 1985. (Ed.). Immobilized Cells and Enzymes. IRL Press, Oxford.

Yim, M.W. and N.F.Y. Tam, 1999. Effects of wastewater-borne heavy metals on mangrove plants and soil microbial activities. Marine Pollution Bulletin, 39: 179-186.

Zahir, Z.A. S.A. Abbas, M. Khaild and M. Arshad, 2000. Substrate dependent microbially derived plant hormones for improving growth of maize seedlings. Pak. J. Bio. Sci., 3: 289-291.

Zantua, M.I. and J.M. Bremner, 1977. Stability of urease in soils. Soil Biol. Biochem., 9: 135-140.