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 vital tested and reported. In plant nutrition their role cannot be substituted 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 physicochemical 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 type 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-glucosides of plant material is necessary (Tabatabai, 1990). Physiological measurements indicate that enzyme-catalyzed reactions in soils have lower activation energies than non-enzyme catalyzed reactions and, therefore, have faster reaction rates (Bromwell 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, 1976 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 peptidase, 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 abiotic 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 phosphatases, suggest that microorganisms seem the logical choice for supplying most of the soil 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 subtillis under certain conditions (Casel and Frewess, 1964) and pyrophosphate and acid phosphatase may exist extracellularly on the surface of cell walls of Saccharomyces mells (Weinberg and Orton, 1963, 1964). Plants have been considered a source of extracellular enzymes in soil. Estermann 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 (1978) demonstrated that sterile corn and soybean roots could exude phosphatase 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 phosphatase 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 (1968) reported that the amount of phosphatase activity in one gram soil was equivalent to 10^7 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 substrates among molecules), and lyases (they catalyze 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.

Seneco and Tabatabai (1986) 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 one hour before assay of aspartase activity showed that the enzyme is stable up to 40°C in field-moist samples and up to 70°C in air-dried samples. Frankenberg and Tabatabai (1986) reported that optimum temperature for soil glutaminase (L-glutaminase 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 65°C. Among the various treatments that affected L-glutaminase activity in soils, autoclaving (121°C, 1 hr), formaldehyde (1mL 5 g-1 soil), dimethyl sulfoxide (1mL 5 g-1 soil) and Na (5mM) reduced the activity by 92, 96, 78 and 14%, respectively. L-Glutaminase activity was greater in untreated soils than in treated soils. Frankenberg and Tabatabai (1986) 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 area than in the A horizon of an adjacent black spruce forest. Stadd 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 1x40 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 variability. In another study, soil samples (top soils 0-20 cm to sub soils 20-40 cm) collected from five of the 15 natural microhabitats in the primary forest, secondary forest, coffee plantation and cultivated plants; showed that the activities of phosphatases, betaglucosidase, and urease were significantly higher in top soils and in most cases in the secondary forests (Salama et al., 1999). Gupta and Bhardwaj (1999) found that both phosphatase and urease activity was greater in grassland and forest soils than uncultivated soils. The findings were in agreement with previous reports that soil enzyme activity is not only affected by the presence of microorganisms, but also by the type of vegetation and management practices. The present study showed that the activity of urease, phosphatase and dehydrogenase enzymes were lower at soil depths in the root zone and interspaces of coconut were greater while in araucan palm only urease and phosphatase activities were greater in the root zone soils. Ifthikar and Khan (1986) found that enzyme activity was decreased with increasing soil salinity (IEC). The decline in enzyme activity (amylase, catalase, urease, phosphatase) with increasing salinity appeared to be associated with changes in osmotic pressure due to saline soil conditions. Certain other soil enzyme activities were significantly higher in soils under junipers (126.6 +/− 3.9 mg p-nitrophenol g−1 soil h−1) than in uncultivated soils (108.6 +/− 4.0 mg p-nitrophenol g−1 soil h−1), and significantly exceeded acid phosphatase activity by a factor of 6. The winter peak in phosphatase activity was higher in a 6.0 MPa soil type than in a 0.5 MPa soil type.
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 (lactic and alkaline phosphatases, β-glucosidase, and arylsulphatase) 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 in three intervals: (1) before, (2) during, and (3) after 10 years of cultivation. Changes in the activities of the soil enzymes were monitored on a semi-annual basis. The results indicated that the activities of specific soil enzymes were significantly influenced by the type of crop rotation, the application of organic amendments, and the management practices employed.

Netzke (1999) reported that microbial properties along four transects from farmland to calcareous grasslands were investigated. It was examined whether changes in microbial 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 the peripheral zone of the calcareous grasslands were found to be significantly lower than in the soils of the central zone. At one site, Galgenberg, the soil respiration and enzyme activities in the peripheral zone were found to be lower than in the central zone, which might be due to a decrease in root mass and an increase in soil microbial biomass (inhibition of phosphatase activity).

Enzymes and the soil environment: Senwo and Tabatabai (1989) conducted a research on the activity of phosphatase in soils, effects of trace elements and its relation-ship to other amido-lyases. The enzyme phosphatase (L-aspartase) and ammonia-lyase, EC 4.3.1.1 catalyzes the hydrolysis of L-aspartate to produce fumarate and NH₃. At 6 mmol L⁻¹ soil, all the trace elements inhibited phosphatase activity in the three field moist soils and their air-dried counterparts. With most of the elements, greater inhibition was found in air-dried than in field-moist soils. Frankenberg and Tabatabai (1991) 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). Paruzzo (1989) revealed that when mussels solid waste compost was added in soil, alkaline phosphomonoesterase, phosphodiesterase and deaminase activity remained constant after reaching maximum values (3-5 months). Arylsulphatase, urease and protease activities returned to baseline after reaching a maximum (2-3 months). Guairen (1989) found that application of wheat and maize straw as manure increased the invertebrate activity of the soil by 40-91 and 160-165 times, respectively, compared with control. The application of compost, pig, horse and cattle manure also increased the activity of invertebrates, but to a lesser extent. The activities of urease and alkaline phosphatase were increased by the application of the manures. Gregorová (1991) revealed that 3 or 4 years after incorporation of fly ash (600 t/ha), activity of soil phosphatase was significantly increased. Pig slurry stimulated phosphatase and urease activity, but decreased phosphatase activity in soils under irrigation. Shendes et al. (1991) investigated that urease and protease 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 matter and microbial biomass.

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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.

Speer et al. (1989) 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 Cd(NO₃)₂ concentrations to account for osmotic effects, and 2) the same range of NO₃ concentrations, comprising NaNO₃ acidified with HNO₃, to account for the acclimatizing 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 acclimatization of all three soils, with the metals which hydrolyze most (Cr, Cu and Pb) having the greatest effect, and the decrease in the most affected soil. 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 metal amendments significantly altered the activity of phosphatase. Sulphatase activity was strongly inhibited by acid and by all metal amendments including Ca. In all three soils, indicating that acclimatization 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. The potential extrapolation 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 phosphorus in the soil. This site has significantly lower enzyme activities of soil acid phosphatase (0.54 µmole p-nitrophenol released g⁻¹ dry weight h⁻¹) and surface acid phosphatase of pine ectomycorrhizas (3.37 µmole p-nitrophenol released g⁻¹ fresh weight h⁻¹) than the control site (1.36 µmole p-nitrophenol released g⁻¹ dry weight h⁻¹ and 12.48 µmole 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 PO₄ and high N: P ratio in pine fine roots from polluted site were found. These 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 (Kleierowska-Rokicka, 1999).

Correlations: Senvo and Tabatabai (1999) found that the activity of aspartase enzyme in soils was significantly correlated with the activities of asparaginase, glutaminase, urease and amylase. The soil properties that related to the amounts of L-glutaminase activities in 26 surface soils included organic carbon and total nitrogen. There was no significant relationship between L-glutaminate activity and pH, percentage of dry or sand. There was, however, a significant correlation between L-glutaminate activity and amylase, 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 Gray Brown Luvisol (Haplustalf). 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 Dusty-Red Latosol (Oxisol) and a Structured Dusty-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 of fraction of P bound 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 organic P, organic P in microbial biomass and higher activity of acid and alkaline phosphatases. The three extractants presented positive and significant correlations with the forms of soil inorganic P and with the plant parameters evaluated.

Olander and Viloushek (2000) measured acid phosphatase and chitinase (N-acetyl-D-glucosaminidase) 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 40 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 increased 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 (NH₄NO₃) were applied to a corn crop (Zea mays L.) at 200 kg N ha⁻¹. Soil enzyme activity (acid phosphatase, dehydrogenase and protease) and CO₂ 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 CO₂ 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 phosphorous substrates. Their activity can be influenced by numerous factors and soil properties. Soil phosphatases play a key role among

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 Dencillo (1993) studied porosity and pore size distribution from thin sections, prepared from undisturbed A 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 were 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-till plots. Root development showed a strict relationship with the presence of smaller pores which were more numerous in no-till 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, which showed a significant correlation 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 (1960) 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 fungi Lycoperdium tristachyum 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 as 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.

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 phosphates 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 amidines, purine and pyrimidine bases, and condensed phosphates; 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. Libberi and Paetott (1982) first demonstrated that indole-3-acetoin and indole-3-acetaldehyde were hydrolyzed in soil to form indole-3-acetic acid (IAA) and indole-3-carboxylic acid, respectively, and Sarvar et al. (1992) reported formation of IAA from tryptophan in soil, and a soil extract was found to have similar activity (Chilivigeac and Mayaudon, 1971). Auxin production was increased in pure culture when rhizobacteria were supplemented with L-tryptophan (Zahir et al., 2000, Asghar et al., 2007).

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 results 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 flasks. Primrose (1978) 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-amino-1-cyclopropane-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 (Curt and Truelove, 1986; Rovira and McDougall, 1987). Sources of activity are intact plant cells on the root surfaces, sloughed off root cells, enzymes secreted by plants or microbes, or active rhizosphere microbes (Dodd et al., 1987). The exploitation of enzymes in the plant root rhizosphere, whether or not extracellular, 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 fir (Pseudotsuga menziesii) seedlings inoculated with the fungus Pisolithus tinctorius (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, 1981). Acid and alkaline phosphatase activities in wheat rhizosphere were strongly correlated with the depletion of organic P (Traffor 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 O2, on the activity of several enzymes has also been investigated by several workers. Waterlogging and an

Reduction caused a 2.5 to 6-fold increase in rhodanase activity in a pottkali acid sulfated soil, but no increases occurred when an alluvial soil was flooded (Ray et al., 1986). A positive correlation between O2 diffusion rate and catalase activity was reported by Glinski and co-workers (1988). After a soil was maintained for 7 days in a waterlogged condition, acid phosphatase, alkaline phosphatase, urease, and arylsulfatase activities were positively correlated with redox potential, whereas phosphodiesterase and amidase were negatively correlated (Pulford and Tabatabai, 1983).

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 histsols to prevent carboxydrase activity and, thus, the mobilization and loss of organic matter from these very productive soils (Mathur et al., 1985; Mathur and Sanderson, 1980). As enzyme components, many metals are also required at low concentrations for optimum biogenesis and catalysis. 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. Zanius and Bremner (1977) has shown 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 the 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 isofenfos and fonofos (Murphy and Minor, 1986; Sikora and Kaufman, 1987). After repeat applications of the insecticides to soil, the activity of a phosphatase, which catalyzed 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. The enzyme activity is then quantified and compared to that of a known substrate (Guggiero 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 types of processes. Filip and Preuss (1985) reviewed the chemical nature of phenols and concluded that these enzymes can protect soils against the accumulation of harmful organic compounds by catalysing reactions involving degradation, polymerization, and incorporation into humic complexes. Enzymatic coupling of certain organic compounds is an important natural process that affects the fate of soil (i.e., reactivity and persistence) in soil and water. (Sarkar et al., 1988; Spald and Sollag, 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 of activity and the effect is only temporary (Dick and Tabatabai, 1983). Heuer and co-authors (1976) reported that adding phosphatase to soil extract containing an organophosphorous insecticide increased the rate at which the insecticide was degraded. Addition of the same enzyme to soil, however, yielded no effect. Peroxidase or laccase effects were enhanced by phosphomonoester compounds in a sandy soil, but the high rates of enzyme activity (50-426 units of activity) and the use of sandy soil made extension of this technique to other soil systems impractical (Shannon and Bart, 1988). Work by Dick and Tabatabai (1989) and Holst 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., 1988) An inherent weakness of immobilized enzymes, however, is their inability to act on water-insoluble substrates. The substrate must diffuse to the immobilized 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; Rosewar et al., 1987; Woodward, 1985). Advantages of using immobilized enzymes for transformation of organic compounds in soil are that 1) it is not necessary to release genetically engineered microorganisms, 2) immobilized enzymes may be reused and are more tolerant of toxic 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-themolecules dependent on pH, ionic strength, temperature and presence or absence of inhibitors; are essential for the solubilization 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 help in the application in soil-plant environment such as an index of soil fertility, indication of pollution, oxidation-reduction status of soil and remediation of contaminated soil.

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