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Role of Soil Enzymes in Nutrient Transformation: A Review

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ABSTRACT: Enzymes are catalytic substances that without undergoing permanent alteration cause chemical reactions to proceed as faster rate. Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system. They are important in catalyzing several important reactions necessary for the life processes of micro-organisms in soils, stabilization of soil structure, and the decomposition of organic wastes, organic matter formation and nutrient cycling. Soil enzymes regulate the functioning of the ecosystem and play key biochemical functions in the overall process of organic matter transformation and nutrient cycling in the soil system. The overall enzyme activity in soil consists of various intracellular and extracellular enzymes that originate from microorganisms (e.g., bacteria, fungi) or from plants and animals. These enzymes may include amylase, arylsulphatases, glycosidase, cellulose, chitins, dehydrogenate, phosphates, protease and urease. Ester sulfates are considered to be the most labile form of soil organic S, they are unavailable to plants and must be hydrolyzed to inorganic SO_4^{2-} before plant uptakes. Arylsulfatase is involved by cleaving the O–S bond and is believed to make a major contribution to the mineralization of ester sulfate in soils. Amylase is a starch hydrolyzing enzyme. It is known to be constituted by α-amylase and β amylase that plays a significant role in the breakdown of starch. Research evidence suggests that several other enzymes are involved in the hydrolysis of starch, but of major importance are α-amylase which converts starch like substrates to glucose and/or oligosaccharides and β-amylase, which converts starch to maltose. β-glucosidase is one such enzyme, being involved in the enzymatic degradation of cellulose, which is the main component of plant polysaccharides. Phosphatases are a broad group of enzymes that are capable of catalysing hydrolysis of esters and anhydrides of phosphoric acid.

Key words: Soil enzymes, nutrient, transformation

INTRODUCTION

Soil is a fundamental resource in the agricultural production system and monitoring its fertility is an important objective in the sustainable development of agro-ecosystems. In order to evaluate soil fertility, changes in its physical, chemical and biological properties must be taken into account. Among the biological features, soil enzymes are often used as index of soil fertility since they are very sensitive and respond to changes in soil management more quickly than other soil variables.

Soil enzyme activities have been suggested as sensitive indicators of soil fertility since they catalyze the principal biochemical reaction that involves nutrient cycles in soil, are very sensitive and respond to changes caused by natural and anthropogenic factors. Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system. They are important in catalyzing several important reactions necessary for the life processes of micro-organisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling (Dick *et al.,* 1994). These enzymes are constantly being synthesized, accumulated, inactivated and/or decomposed in the soil, hence playing an important role in agriculture and particularly in nutrients cycling. The activities of these enzymes in soils undergo complex biochemical processes consisting of integrated and ecologically-connected synthetic processes, and in the immobilization and enzyme stability. In this regard, all soils contain a group of enzymes that determine soil metabolic processes which, in turn, depend on its physical, chemical, microbiological and biochemical properties. The enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amounts of organic matter content, composition and activity of its living organisms and intensity of the biological processes (Stevenson, 1986).

In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes and variable substrates that serve as energy sources for micro organisms (Kiss *et al.,* 1978). These enzymes may include amylase, arylsulphatases, glycosidase, cellulose, chitins, dehydrogenate, phosphates, protease and urease released from plants (Miwa *et al.,* 1937), animals, organic compounds and micro-organisms .A better understanding of the role of this soil enzymes activity in the ecosystem will potentially provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management practices. Studies indicate that high enzyme activity signals mineral element limitation in the ecosystem. Although there have been extensive studies on soil enzymes little has been reported on their roles in agricultural development.

Soil enzymes regulate the functioning of the ecosystem and play key biochemical functions in the overall process of organic matter transformation and nutrient cycling in the soil system. The overall enzyme activity in soil consists of various intracellular and extracellular enzymes that originate from microorganisms (e.g., bacteria, fungi) or from plants and animals (e.g., plant roots or residues, digestive tracts of small animals). The same enzyme can originates from different sources and the exact origin as well as the temporal and spatial variability of the activity is difficult to identify. .Intracellular enzymes exist in different parts of living and proliferating cells, while extracellular enzymes are produced and secreted by living cells and act outside the parent cells as free enzymes in a soil solution or as enzymes that are still associated with the external surface of the root epidermal or microbial cell wall (so-called ectoenzymes).

When secreted outside the cell, enzymes can be free in a soil solution or they can be absorbed by soil mineral constituents or complexed with humic substances or both. The amount of free extracellular enzymes in soil is very low compared with that in the adsorbed state due to their short life span in an inhospitable environment. Adsorbed enzymes are resistant to proteolysis, thermal and chemical denaturation, but immobilization usually protects enzymes against degradation at the cost of some loss of activity. Although bounded enzymes reveal less activity than free enzymes, the most important part of their activity is being responsible for the transformation of organic matter and the availability of nutrients (Ganeshamurthy *et al.,* 1995).

Numerous factors can influence enzyme activity in soil. Natural parameters (e.g., seasonal changes, geographic location, in situ distribution, physical-chemical properties, content of organic matter and clay) usually affect the enzyme activity level by influencing both the production of enzymes by plants and microorganisms and their persistence under natural conditions.

The physical and chemical properties of a soil are involved in the immobilization and stabilization processes of most extracellular enzymes. A high content of clay or humus colloids is usually associated with stable but less active enzymes. Agricultural activities and environmental pollution (e.g., fertilizers, pesticides, tillage, heavy metals, PAHs) may affect the chemical composition and structural characteristics of soil, which in turn will influence the species composition and abundance of soil microorganisms, their metabolic activity, the enhancement or suppression of enzyme production and the overall activity of an enzyme in soil. Soil enzymes are important in soil functioning because of the following features:

1. They play a critical role in the decomposition of organic materials and the transformation of organic matter,
2. They release available nutrients to plants,
3. They participate in N_2 fixation, nitrification and denitrification processes, and
4. They take part in the detoxification of xenobiotics, such as pesticides, industrial wastes, etc.

ROLE OF SOIL ENZYMES

Table 1: Role of soil enzymes.

Enzyme	Organic Matter Substances Acted on	End Product	Significance	Predictor of Soil Function
Beta-glucosidase	Carbon compounds	Glucose (sugar)	Energy for microorganisms	Organic matter decomposition
FDA hydrolysis	Organic matter	Carbon and various nutrients	Energy and nutrients for microorganisms, measure Microbial biomass	Organic matter decomposition nutrient cycling
Amidase	Carbon and nitrogen compounds	ammonium (NH_4)	Plant available NH4	Nutrient cycling
Urease	Nitrogen (urea)	Ammonia (NH_3) and carbon dioxide (CO_2)	Plant available NH4	Nutrient cycling
Phosphatase	Phosphorus	Phosphate (PO_4)	Plant available P	Nutrient cycling
Sulfatase	Sulfur	Sulfate (SO_4)	Plant available S	Nutrient cycling

A. Amylase

Amylase is a starch hydrolyzing enzyme (Ross, 1976). It is known to be constituted by α-amylase and β-amylase. Studies have shown that α-amylases are synthesized by plants, animals and micro-organisms, whereas, β-amylase is mainly synthesized by plants (Pazur, 1965). This enzyme is widely distributed in plants and soils so it plays a significant role in the breakdown of starch. Research evidence suggests that several other enzymes are involved in the hydrolysis of starch, but of major importance are α-amylase which converts starch like substrates to glucose and/or oligosaccharides and β-amylase, which converts starch to maltose (Thoma *et al.,* 1971).

Studies have, however, indicated that the roles and activities of α-amylase and β-amylase enzymes may be influenced by different factors ranging from cultural practices, type of vegetation, environment and soil types (Rose and Roberts, 1970). For example, plants may influence the amylase enzyme activities of soil by directly supplying enzymes from their residues or excreted compounds, or indirectly providing substrates for the synthetic activities of micro-organisms. Greater understanding the role and other chemical, biological, physical and agronomic factors influencing the functioning of amylase enzymes in the soil that will further define the significance of these enzymes in the soil, and enable proper management techniques to be devised to maximize the benefits that may be derived from such enzymes.

B. Arylsulphatases

The relative proportions of inorganic and organic S forms in soils vary widely. However, the organic S compounds are dominant and a large proportion of the organic S is present as ester sulfates. Although ester sulfates are considered to be the most labile form of soil organic S, they are unavailable to plants and must be hydrolyzed to inorganic SO_4^{2-} before plant uptake.

In this process arylsulfatase is involved by cleaving the O–S bond and is believed to make a major contribution to the mineralization of ester sulfate in soils (Tabatabai, 1994).

Arylsulphatases are typically widespread in nature (Dodgson *et al*., 1982) as well as in soils. They are responsible for the hydrolysis of sulphate esters in the soil and are secreted by bacteria into the external environment as a response to sulphur limitation (McGill and Colle, 1981). Its occurrence in different soil systems is often correlated with microbial biomass and rate of S immobilization. The role of this enzyme in the hydrolysis of aromatic sulphate esters (R- O-SO_3^-) to phenols (R-OH) and sulphate, or sulphate sulphur (SO_4^{-2} or SO_4-S) is shown in the following simple chemical equation (Tabatabai, 1994).

$$R-O-SO_3^- \xrightarrow[Arylsulphatases]{Hydrolysis} R-OH + SO_4^{-2}$$

The enzyme was first detected in soils by Tabatabai and Bremner (1970a) and since then a lot of investigations have been conducted taking environmental factors into consideration that may influence its activity. Soil arylsulfatase activity has a broad pH optimum from pH 5.8 to 8.2 (Klose *et al.,* 1999). Thus, under almost neutral or slightly alkaline conditions, enzyme activity should be higher as compared to acid soils. However, release from roots may result in a decrease in rhizosphere pH and therefore in a reduction of the enzyme activity. Since the microbial biomass is considered to be the primary source of enzymes in soils and the immediate vicinity of roots is generally recognized to be an environment that promotes the development of a large population of soil microbes.

This inferred the reduction of enzyme activity at low pH, brought about by proton excretion, may be over-compensated by the higher metabolic activity of the microbial biomass. This compensation was clearly shown in the soil supplied with compost for many years where microbial parameters were influenced by soil organic matter content, resulting in higher enzyme activity. There existed a strong correlation between humus content and arylsulfatase activity. Therefore, it may be assumed that the long-term amendments of FYM and compost, respectively, not only increase the organic matter and total S content of soils, but also the sulfatase activity that indicated the agronomic measures such as compost and FYM application, improve the availability of soil organic S in the rhizosphere with the age of the plants, which is attributed to a gradual build-up of a microbial population close to the roots.

Considering the importance of S in plant nutrition, a better understanding of the role of arylsulphatases in S mobilization in agricultural soils is critical. So far, very little is known about specific microbial genera or species that play an important role in the soil organosulphur circle (Kertesz and Mirleau, 2004) in which arylsulphatases is the key enzyme.

During the last two decades, sulfur deficiency was observed particularly in fields distantly located from industrial plants and later on, as well on fields in the narrow surroundings of those plants. Increasing sulfur deficiency conditions are thought to have several reasons, e.g. the use of sulfur-free synthetic fertilizers, cultivation of crops with high sulfur requirement and reduction of SO_2 emission as a result of new industrial technologies.

In most aerobic soil types ester sulfates account for up to 70% of the organic S and therefore are the most important organic S reserve in soils. Their mineralization occurs through sulfatases originating from microorganisms (Kertesz, 1999).

Hydrolysis of aromatic ester sulfates is catalyzed by periplasmically located arylsulfatases cleaving sulfate from the organic moiety of the molecules. The enzyme activity in soils depends on diverse parameters, such as the presence of sulfur anions (Dodgson *et al.,* 1982), seasonal variations in soil moisture (Cooper, 1972; Freeman *et al.,* 1996), pH (Tabatabai and Bremner, 1970b) and heavy metal abundance. According to Klose *et al.* (1999) arylsulfatase activity correlates with the amount of organic carbon and the activity decreases with depth in profile as organic matter decreases (Tabatabai and Bremner, 1970a). Recent investigations of Deng and Tabatabai (1997) revealed that tillage and residue management have significant effects on arylsulfatase activity in soils.

Therefore it may be assumed that agronomic measures such as the long-term application of farmyard manure or compost have an impact on the enzyme activity. Furthermore it is expected that crop species may influence the activity of arylsulfatase, being highest with Cruciferous with their high S demand.

Arylsulfatases are common enzymes as well in mammals and invertebrate, but in higher plants their existence is still a matter of debate. There observed a low arylsulfatase activity in seeds of *Sinapis niger*, whereas with *Sinapis album* seeds no activity was found. It is however, not clear whether sterile or non-sterile plant material was used in their studies. In studies with tussock grass species (*Festuca novae-zealandiae, Poa colensoi, Chionochloa rigida*, *C. flavescens, C. macra, C. rubra*), grown under non-sterile conditions the following ranking in arylsulfatase activity was given: leaves /litter /roots /soil. Because enzyme activity increased with increasing numbers of microorganisms. In *Cucumis sativus* root tips arylsulfatase activity might be located within the ER and at the nucleus. However, it seems questionable whether arylsulfatase activity with the described localization would be able to cleave substrates present in the soil.

C. β-glucosidase

Of the extracellular enzymes in soils, those involved in the degradation of soil organic matter are of particular interest. β-Glucosidase is one such enzyme, being involved in the enzymatic degradation of cellulose, the main component of plant polysaccharides. Cellulose consists of polymer chains of β-1,4, linked glucose units and its enzymatic degradation initiated by endo β -1,4-glucanase, which breaks cellulose chains into smaller units, and cellobiohydrolase, which cleaves the dimer cellobiose (two β -1,4 linked glucose units) from the reducing 'ends' of the molecules. β-Glucosidase completes the hydrolysis process by catalyzing the cleavage of cellobiose to release two moles of glucose per mole of cellobiose and, therefore, regulates the supply of an important energy source for microorganisms unable to directly take up cellobiose. Indeed, β -glucosidase activity may be the rate-limiting step in cellulose degradation (Alef and Nannipieri, 1995). β-Glucosidase is derived predominantly from soil microbial heterotrophs, in particular members of the mucorales (fungi), such as *Actinomucor* or *Mortierella* (Hayano and Tubaki, 1985). Its synthesis in such organisms is induced by the products of cellulose breakdown, including cellobiose, glucose.

β-Glucosidase activity may be a particularly useful enzyme for soil quality monitoring because of its central role in soil organic matter cycling, which is generally regarded as an important component of soil quality. B-Glucosidase is characteristically useful as a soil quality indicator, and may give a reflection of past biological activity, the capacity of soil to stabilize the soil organic matter, and can β glucosidase enzyme is very sensitive to changes in pH, and soil management practices.

Tabatabai (2000) reported β-glucosidase as sensitive to pH changes. This property can be used as a good biochemical indicator for measuring ecological changes resulting from soil acidification in situations involving activities of this enzyme. β-glucosidase enzyme is also known to be inhibited by heavy metal contamination such as Cu and several others (Wenzel *et al.,* 1995). For instance, studies have shown that plant debris did not decomposed or show β-glucosidase activities when exposed to heavy metal polluted soils (Geiger *et al*., 1993).

The reaction of the analysed soil ranged from slightly acid to neutral. Changes in the soil reaction were caused by neither the increasing rates of ammonium nitrate nor the various combinations of P, K, Ca, and Mg and S fertilisation. The changes in the soil reaction were to a greater extent caused by the crops grown than nitrogen fertilisation. The content of sulphur available to plants depends on the soil reaction. When exposed to a higher soil pH, more sulphur is released owing to a higher rate of organic matter decomposition; when the soil pH is lower, an increased adsorption of sulphates on hydrate iron and aluminium oxides as well as kaolinite occurs. Similarly, the activity of soil enzymes depends on the concentration of hydrogen ions in soil. Arylsulphatase has a broad pH optimum from 5.8-8.2 (Klose *et al*. 1999).

The content of total sulphur in soil ranged from 0.038 g kg^{-1} to 0.059 g kg^{-1}. It was indicated that the content of S in mineral soils under agricultural use ranged from 0.07 to 1.07 g kg^{-1}. In the examined soil, the content of total sulphur was found below the range characteristic for mineral soils in Poland's agricultural areas. According to Yang *et al*. (2007) and Kotkova *et al.* (2008), the total soil sulphur depends on the type of fertilisers applied (mineral or organic). In our experiment, both types of fertilisation, with nitrogen and with the other macro elements, affected the content of sulphur in the soil. In the plots receiving either NK or NPKS fertilizers for more than 80 years, no significant accumulation of total sulphur, as compared with the control, could be detected.

The highest total sulphur content was observed in the soil collected from the treatments with the highest rate of ammonium nitrate (200 kg ha^{-1}). The other nitrogen fertiliser rates did not show any significant effect on the content of this macro element in soil.

D. Cellulases

Cellulose is the most abundant organic compound in the organisms; cellulose in plant debris has to be degraded into glucose, cellobiose and high molecular weight oligosaccharides by cellulases enzymes. Cellulases are a group of enzymes that catalyze the degradation of cellulose; polysaccharides build up of β -1, 4 linked glucose units (Deng and Tabatabai, 1994). It has been reported that cellulases in soils are derived mainly from plant debris incorporated into the soil, and that a limited amount may also originate from fungi and bacteria in soils (Richmond, 1991). Currently, it is generally accepted that the cellulases system comprises of three major types of enzymes. They include: endo-1, 4-β-glucanase which attacks the cellulose chains at random, exo-1, 4-β-glucanase which removes glucose or cellobiose from the non-reducing end of the cellulose chains, and -D-glucosidase which hydrolyses cellobiose and other water soluble cellodextrins to glucose. Previously, several hypotheses were proposed about the mechanisms involved in the degradation of cellulose by the cellulases (Wood, 1991) although none of them has been fully accepted.

Demonstrating the effects of increasing concentrations of fungicides on cellulases activities, Petkar and Rai (1992) showed that there was a decreasing effect with fungicides captan, cosan, thiram, zinels and sandolex. More recently, Arinze and Yubedee (2000) reported that fungicides benlate, calixin and captan inhibited cellulase activity in *Fusarium monoliforme* isolates. Captatol inhibited cellulose activity in the sandy loam soil and chlorothalonil showed a clear reduction in cellulase activity under flooded or non-flooded conditions.

Studies have shown that activities of cellulases in agricultural soils are affected by several factors. These include temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical structure of organic matter and its location in the soil profile horizon, quality of organic matter/plant debris and soil mineral elements and the trace elements from fungicides. Srinivasulu and Rangaswamy (2006) reported a significantly more stimulatory effect of cellulases in black soil than red soil. Several mechanisms have been proposed in the degradation of cellulose by cellulases.

For instance, chitin in the presence of cellulose induces the synthesis of chitinase and other cell wall lytic enzymes which promote the release of the β - glucosidase into the medium.

All these findings suggest that activities of cellulases can be used to give preliminary indication of some of the physical chemical properties of soil, thus, easing agricultural soil management strategies. Since cellulases enzymes play an important role in global recycling of the most abundant polymer, cellulose in nature, it would be of critical importance to understand this enzyme better so that it may be used more regularly as a predictive tool in our soil fertility programmes.

E. Dehydrogenase

Soil dehydrogenases are the major representatives of the Oxidoreductase enzymes class. Among all enzymes in the soil environment, dehydrogenases are one of the most important, and are used as an indicator of overall soil microbial activity (Salazar *et al.,* 2011), because they occur intracellular in all living microbial cells (Moeskops *et al.,* 2010; Zhao *et al.,* 2010; Yuan & Yue, 2012). Moreover, they are tightly linked with microbial oxidoreduction processes that are important dehydrogenases do not accumulate extracellular in the soil.

Dehydrogenases play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors. Many specific dehydrogenases transfer hydrogen to either nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate (Subhani *et al.,* 2001).

Brzezińska *et al.* (1998) found that active dehydrogenases can utilize both O_2 and other compounds as terminal electron acceptors, although anaerobic microorganisms produce most dehydrogenases. Therefore, DHA reflects metabolic ability of the soil and its activity is considered to be proportional to the biomass of the microorganisms in soil.

However, the relationships between an individual biochemical property of soil DHA and the total microbial activity is not always obvious, especially in the case of complex systems like soils, where the microorganisms and processes involved in the degradation of the organic compounds are highly diverse.

F. Phosphatases

Phosphatases are a broad group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid.

In soil ecosystems, these enzymes are believed to play critical roles in P cycles as evidence shows that they are correlated to P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system (Dick *et al.,* 2000).

According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, they can be classified as phosphoric monoester hydrolases or phosphomonoesterases (EC 3.1.3), phosphoric diester hydrolases or phosphodiesterases (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5) and enzymes acting on phosphoryl-containing anhydrides (EC 3.6.1) and on P–N bonds (EC 3.9). Phosphatases can also be subdivided according to their regulation (e.g. calmodulin), the requirements of metal cations for their activity (e.g. Mg_2þ and Ca_2þ) and their sensitivity to various phosphatase inhibitors.

Phosphomonoesterases include acid and alkaline phosphomonoesterase (which hydrolyse monoester bonds including mononucleotides and sugar phosphates), phosphoprotein phosphatases (which hydrolyse phosphoester bonds of phosphoserines, phosphothreonines or phosphotyrosines), phytases (EC 3.1.3.26 for 4-phytase and EC 3.1.3.8 for 3-phytase, which hydrolyse all six phosphate groups from inositol hexaphosphate) and nucleotidases. Acid and alkaline phosphomonoesterases do not hydrolyse phosphates of phytic acid (myo-inositol hexaphosphates) but they can hydrolyse lower-order inositol phosphates (Cosgrove 1980). Phosphodiesterases hydrolyse one or two ester bonds in phosphodiester compounds and include nucleases, which catalyse the hydrolysis of phosphodiester bonds of nucleic acids to produce nucleotide units or mononucleotides but not inorganic phosphates. Phospholipases hydrolyse phospholipids. We shall also discuss inorganic pyrophosphatase, the enzyme that hydrolyses pyrophosphate to inorganic P, because pyrophosphate can be used as a fertilizer (Dick and Tabatabai 1978).

Acid phosphomonoesterase activities in soil have been frequently measured at pH 6.5; however, at this pH the measured enzyme activity may include acid and alkaline phosphomonoesterase activity (Malcom 1983). Acid phosphomonoesterase activity generally prevails in acidic soils, whereas alkaline phosphomonoesterase activity prevails in alkaline soils, and for this reason the activities of the two enzymes are negatively correlated phosphodiesterase and phosphomonoesterase activities may act sequentially. Pant and Warman (2000) observed that acid phosphomonoesterase (from wheat germ), alkaline phosphomonoesterase (from calf intestinal mucosa), phospholipase (from Clostridium perfingrens) and nuclease (from Staphylococcus aureus), all immobilised on positively charged supports, were able to mineralise (at pH 7.0) organic P extracted from different soils by water or NaOH. The activities of both phosphomonoesterases were generally increased when these enzymes were used with one of the two phosphodiesterases.

Soil acid phosphomonoesterase activity was higher at low inorganic P content of soil than at high content, and the enzyme activity of the low-P soil was significantly correlated with herbage yield, probably due to the importance of organic P mineralization for plant P nutrition. Higher enzymatic hydrolysis of organic P depended on the higher microbial P immobilisation but not on the higher mineralisation of organic P compounds. Application of inorganic P can repress the synthesis of phosphomonoesterases in soil and, indeed, phosphate inhibits the phosphatase activities of soil.

However, the absence of a response of phosphatase activities to P addition has also been reported. For example, the application of triple superphosphate to an oak soil in 1992 did not affect acid phosphomonoesterase activity of soil samples taken in 1993 and 1994 (Schneider *et al.* 2001).

Addition of phosphate with glucose and inorganic N did not stimulate the phosphomonoesterase activity (pH 6.5) of soil, whereas the stimulation occurred in the respective soil treated only with glucose and inorganic N (Nannipieri *et al.* 1978).

Presumably, the enzyme activity was not decreased by phosphate due to the presence of extracellular phosphomonoesterases stabilised by soil colloids or due to the presence of constitutive microbial phosphomonoesterase in soil. Enzyme assays discriminating the activities of extracellular stabilized enzymes from activities of enzymes associated with soil microorganisms would permit an understanding of the underlying mechanisms.

Phosphatase Activities of Bulk and Rhizosphere Soil and the Origin of Phosphatases in Soil. It is well established that enzyme activities are higher in rhizosphere than bulk soil (Both acid and alkaline phosphomonoesterase activities of soil were increased near the rhizoplane of *Brassica oleracea, Allium cepa, Triticum aestivum* and *Trifolium alexandrium* and such an increase depended on plant species, soil type and plant age (Tarafdar and Jungk 1987). Probably, the increase with plant age was due to the gradual formation of the rhizosphere microflora and to the release of plant phosphomonoesterases. The distance from the rhizoplane at which the rhizosphere effect on enzyme activities was observed was higher for acid (from 2 to 3.1 mm) than for alkaline (from 1.2 to 1.6 mm) phosphomonoesterase. There was an inverse and significant correlation between the acid or the alkaline phosphomonoesterase activity and the content of organic P of the rhizosphere soil sampled from *Triticum aestivum* and *Trifolium alexandrium,* whereas the content of inorganic P increased towards the rhizoplane. Obviously, it is difficult to interpret the measurement of phosphomonoesterase activities of rhizosphere soil if the contribution of plant and microbial phosphatases to the measured enzyme activity are not separated. Colvan *et al*. (2001) suggested that acid phosphomonoesterase activity of hay meadow soils was due to enzyme released by plants, because the enzyme activity was high and microbial P was low in soils never treated with fertilizer or treated with N or K fertilizer for 100 years. However, the measured acid phosphomonoesterase activities could also have been at least partly due to enzymes synthesized by the soil microflora in response to P-deficient conditions (Nannipieri 1994).

Effects of Soil Handling and Soil Properties on Soil Phosphatase activities. Both air-drying and freeze-drying often decrease acid and alkaline phosphomonoesterase activity of soil. However, Eivazi and Tabatabai (1977) found an increase in acid phosphomonoesterase and phosphotriesterase activities and a decrease in alkaline phosphomonoesterase and phosphodiesterase activites after air-drying of soil. Acid phosphomonoesterase activities in moist soils stored at 4°C and in the respective air-dried soils were significantly correlated (Baligar *et al.* 1988). Air-drying also decreased pyrophosphatase activity of soil, and the best storage conditions were to keep field-moist soils at 5°C (Tabatabai and Dick 1979). Probably the best strategy is to keep moist soils at 4ºC and measure the enzyme activity as soon as possible. Kandeler (2007) suggests that if the determination of the enzyme activity requires storage periods longer than 3 weeks at 4ºC, it is better to store the samples at 20ºC than at 4ºC. At the end of the storage period, soil samples are allowed to thaw at 4ºC for about 2 days before the determination of the enzyme activity.

Steam sterilisation at 121°C for 1h completely inactivated alkaline phosphomonoesterase, phosphodiesterase and phosphotriesterase activity, but increased acid phosphomonoesterase activity. Heating above 60°C inactivated the pyrophosphatase activity of soil (Tabatabai and Dick 1979). It is well established that phosphatase activities are correlated with the content of organic matter and decrease with soil depth.

The amount of acid phosphatase exuded by plant roots has been shown to differ between crop species and varieties, as well as crop management practices. For instance, research has shown that legumes secrete more phosphatase enzymes than cereal (Yadav and Tarafdar, 2001). This may probably be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals. In their studies, Li *et al.* (2004) reported that chickpea roots were also able to secrete greater amounts of acid phosphatase than maize. The ability to solubilise soil mineral elements by these phosphomonoesteraces is expected to be a higher in biologically-managed systems because of a higher quantity of organic C found in those systems.

Effects of Pollutants on Soil Phosphatase Activities. Both acid and alkaline phosphomonoesterase activities have been monitored to evaluate the effects of several pollutants on organic P mineralization in soil. The ecological dose (ED50), i.e. the concentration of the pollutant that reduces the enzyme activity by 50%, has been calculated to quantify some of these effects. Acid phosphomonoesterase activity of a blanket peat, an organic grassland soil and a calcareous grassland soil were high due to the P limitation induced by long term atmospheric nitrogen deposition (Turner *et al.* 2002b), whereas sulfur pollution decreased the acid.

Short-term laboratory incubations might not reflect the toxic effects in long-term heavy metal polluted soils. Alkaline phosphomonoesterase activity was still reduced in soils contaminated with Cd (concentration ranging from 0 to 0.36 m mol Cd kg^{-1}) in 1988–1990 and sampled in 2001, despite very low Cd availability, as determined by water extraction (Renella *et al.* 2004). In contrast, acid phosphomonoesterase activity and the composition of the bacterial community, determined by plate counts, were unaffected, probably because the Cd pollution caused physiological adaptations rather than the selection of metal-resistant culturable bacteria. Addition of dry milled ryegrass to these long-term Cd-contaminated soils increased both microbial biomass and acid and alkaline.

In fact, the activity of acid and alkaline phosphatases was found to correlate with organic matter in various studies (Aon and Colaneri, 2001). Another factor that influences the rate of synthesis, release and stability of this enzyme is the soil pH. For example, phosphomonoesteraces inducibility and their exudation intensity by plant roots and micro-organisms are determined by their orthophosphate need, which is in turn affected by soil pH. It is, therefore, anticipated that management practices that induce P stress in the rhizosphere may also affect the secretion of these enzymes in the ecosystem.

To date, there have been few studies examining the influence of management options in the ecosystem on phosphatases activity in soil where most crops are grown. Understanding the dynamics of enzyme activities in these systems is crucial for predicting their interactions as their activities may, in turn, regulate nutrient uptake and plant growth.

Intercropping is becoming more and more important to increase crop productivity to meet food demands of an increasing population, especially in the northwest China (Li *et al.,* 1999). Intercropping, through effective use of water, nutrients and solar energy, can significantly enhance crop yields compared with monoculture cropping (Willey, 1990; Morris and Garrity, 1993). When two crops are planted together, interspecific competition or facilitation between plants may occur (Vandermeer, 1989). For example, mixtures of cereals and lupins produced higher grain yields than either crop grown alone; the yield increases were not only due to improved nitrogen nutrition of the cereal component, but also to other unknown causes (Nel, 1975).

Maize yield was increased by intercropping with groundnut, mainly because of an enhanced P uptake (El Dessougi *et al.,* 2003). White lupin (*Lupinus albus*) exuded organic acids to mobilize sparingly soluble phosphate which made more P available for wheat than when it was grown as a monoculture. Pigeon pea increased P uptake of the intercropped sorghum by exuding piscidic acid that chelates Fe^{3+} and subsequently releases P from $FePO_4$. In a field experiment, faba bean facilitated P uptake by maize (Li *et al.,* 2003b). However, all these studies were focused on inorganic P in the soil. In most agricultural soils, organic P comprises 30–80 % of the total P. The largest fraction of organic P, approx. 50 %, is in the form of phytin and its derivatives. Organic P sources can be utilized by the plant after they are hydrolysed by phosphatase (Gilbert and Knight, 1999). Agroforestry species with high acid phosphatase activities can mobilize and utilize organic P in the soil (George *et al.,* 2002a). Chickpea could facilitate P uptake by associated wheat from an organic P source. The mechanism involved, however, remains unclear. The objectives of this study were to quantify acid phosphatase excreted from chickpea roots and to examine whether acid phosphatase contributed to the facilitation of P uptake of intercropped maize that had been supplied with phytate.

The experiment consisted of three P treatments and three treatments of root separation between maize and chickpea. The three P treatments were (1) no added P, (2) $Ca(H_2PO_4)2\_H_2O$ (orth-P), 50 mg P kg soil (calcium salt was used to avoid adding extra potassium to the soil), and (3) phytate-Na, 50 mg P kg^{-1} soil.

Three root separations were (1) plastic sheet to eliminate root contact and solute movement, (2) nylon mesh (30 mm) to prevent root contact but permit solute exchange, and (3) no root separation (Fig. 1). Plastic pots (0_15 m diameter) were cut in the middle, separated into two compartments and then reconstructed. Each compartment of the pot was filled with 1.5 kg of air-dried and sieved (2 mm) soil. The soil contained 4.2 g organic matter, 0.3 g total N, 1.7 mg $NaHCO_3$-extractable P and 48.9 mg K per kg soil, and had a pH (extracted by solution of 0.01 mol $LCaCl_2$, with soil: solution of 1: 5) of 7.8. Basal nutrients (without P) were added in solution to soil.

The soil was thoroughly mixed by shaking. Six germinated seeds of chickpea and four seeds of maize were grown in the pot (one species in each compartment). Plants were thinned to five per compartment for chickpea and to two for maize 10 days after sowing. The pots were watered daily to field capacity (16 %, w/w).

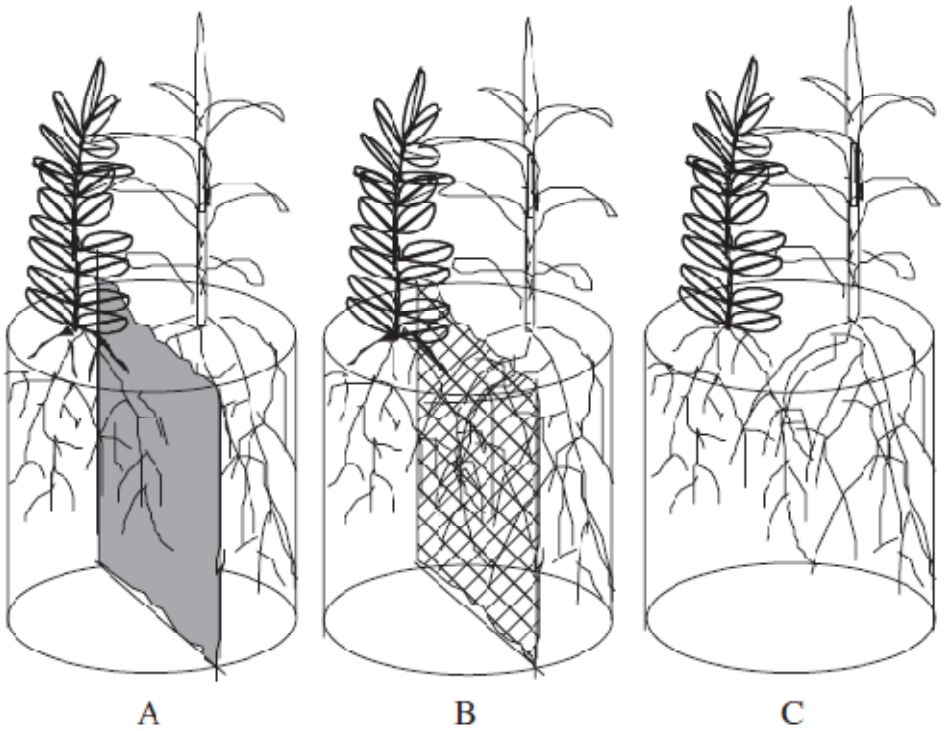

Fig. 1. (A) Schematic diagram illustrating separating the root system of maize and chickpea by a solid root barrier plastic sheet, (B) nylon mesh and (C) no root barrier.

Harvesting and soil sampling

After transplanting for 15 d in nutrient solution, one plant of maize and two plants of chickpea were sampled from each container every 5 d. Plant roots were thoroughly washed with distilled water for assay of acid phosphatase activity. Dry weights of roots and shoots were recorded. Phosphorus concentration in plant tissues was determined using the vanodomolybdate method after the plant material was digested in concentrated H_2SO_4.

The plants were harvested 40 d after sowing. Roots were separated from soil; any soil remaining on the surfaces of roots was brushed off and the soils placed immediately in a cold room at 4°C. Dry weights and P concentrations in shoots were measured.

Soil acid phosphatase activity in the rhizosphere had a similar trend to that of plant roots n the hydroponic culture but, the magnitude of increase was much lower. Average soil acid phosphatase activity (combining the data from all root barrier treatments) in the rhizosphere of chickpea roots supplied with phytate was twice.

Acid phosphatase activity of plant roots supplied with organic P or without P was significantly enhanced compared with those grown in inorganic P. Average acid phosphatase activity of intercropped and monoculture maize supplied with phytate or no P addition was 81% and 62% greater than that supplied with KH_2PO_4 at Day 20, respectively. Average acid phosphatase activity secreted from intercropped and monoculture chickpea roots supplied with phytate was 30-fold greater than maize at Day 20. Similar results for maize and chickpea were observed at Day 15 and Day 25, respectively. Compared with maize, the magnitude of increase in acid phosphatase activity of chickpea was less than that of maize at Day 15 but was greater than that of maize at later days.

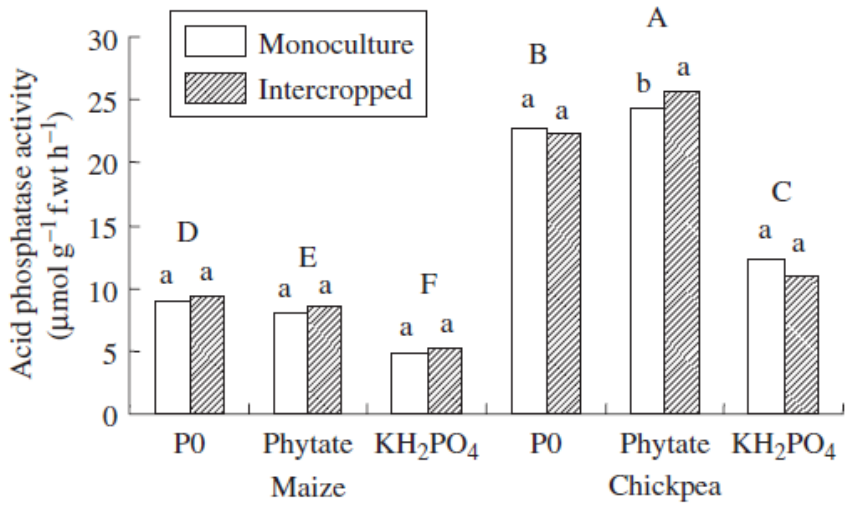

Fig. 2. Acid phosphatase secreted by the roots of maize and chickpea that had received phytate-P,KH_2PO_4-P or no P addition at Day 20 in hydroponics.

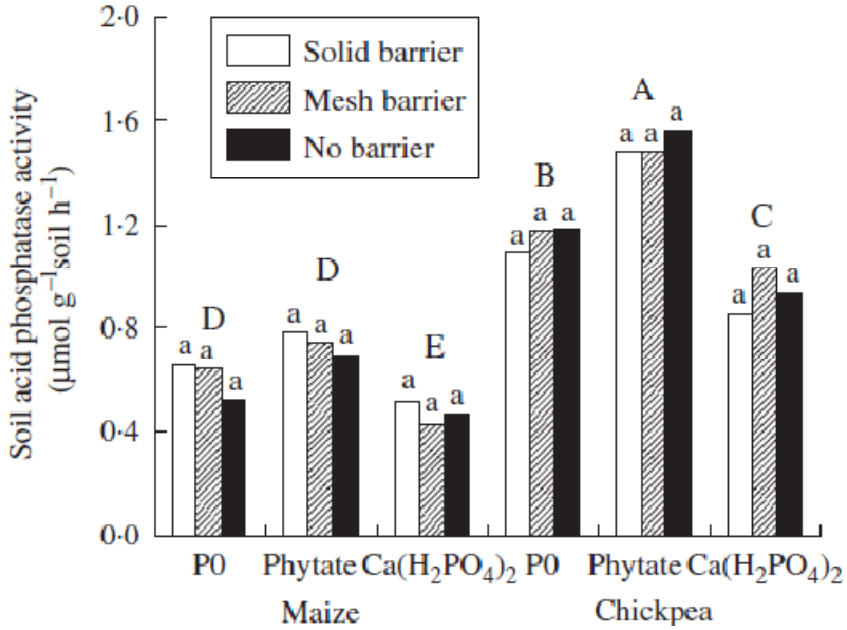

Fig. 3. Acid phosphatase secreted by the roots of maize and chickpea that had received phytate-P, KH_2PO_4-P or no P addition at Day 20 in hydroponics.

G. Urease

Urease enzyme is responsible for the hydrolysis of urea fertilizer applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH. This, in turn, results in a rapid N loss to the atmosphere through NH_3 volatilization. Often, urea is the main source of N in many crops including flooded or irrigated rice and maize in many parts of Africa and Asia. Despite the importance of this fertilizer, its efficiency has been reported as low due to substantial N lost to the atmosphere through volatilization, a process mediated by the urease enzyme.

Soil urease originates mainly from plants and micro-organisms found as both intra- and extra-cellular enzymes. The stability of this enzyme in the system is affected by several factors. For example, studies have shown that extracellular urease associated with soil organo-mineral complexes is more stable than urease in the soil solution and those humus-urease complexes extracted from soil are highly resistant to denaturing agents such as extreme temperatures and proteolytic attack. On the other hand, urease extracted from plants or micro-organisms is rapidly degraded in soil by proteolytic enzymes. This suggests that a significant fraction of ureolytic activity in soil is carried out by extracellular urease, which is stabilized by immobilization.

Since urease plays a vital role in the hydrolysis of urea fertilizer, it is important to uncover other unknown factors that may reduce the efficiency of this enzyme in the ecosystem. A better understanding of this enzyme would provide more effective ways of managing urea fertilizer especially in high rainfall areas, flooded soils and irrigated lands as well as where urea fertilizer is vulnerable to urease enzyme.

Urea hydrolysis. In soil, urea is decomposed enzymatically to CO_2, and NH_3. Effects of various treatments on urea decomposition activity in soil can often be related to those obtained with pure urease. Therefore, it has been assumed, probably correctly, that the reaction is catalyzed by soil urease.

The currently accepted reaction mechanism of enzymatic hydrolysis of urea is the enzymatic cleavage of urea to ammonia and carbamic acid, followed by the chemical hydrolysis of carbamic acid to ammonia and carbon dioxide.

$$\underset{\text{Urea}}{NH_2CONH_2} + \underset{\text{Water}}{H_2O} \dashrightarrow \underset{\text{Ammonium Carbamate}}{NH_2COONH_4}$$

$$\underset{\text{Ammonium Carbamate}}{NH_2COONH_4} \dashrightarrow \underset{\text{Ammonia}}{2\,NH_3} + \underset{\text{Carbon Dioxide}}{CO_2}$$

Urease hydrolysis, as in any enzymatic reaction, may only be needed to reduce activation energy for the formation of the intermediate product. Even in the absence of enzymes, urea can be hydrolysed physico-chemically. However, chemical hydrolysis is very slow compared to biochemical enzymatic hydrolysis. Therefore, it can be concluded that urea hydrolysis in soils is mainly brought about by the action of the enzyme, urease.

Origin and locus of soil urease

Origin. Urease activity in soil may originate from plant residues, animal waste or soil microbes. Plants are rich sources of ureaese. However, there is no direct evidence for the production of urease by plant roots. Therefore, the addition of plant materials and animal wastes may supply urease to the soil. Soil urease is of microbial origin. Most of the nitrosomonas and nitrosospira isolated from soils in Scotland were capable of hydrolysing urea. The ability to hydrolyse urea was found to vary from 17 to 77% for soil bacteria, and from 78 to 98% for soil fungi Urease has been purified from certain bacterial species and the factors affecting the production of urease by microorganisms has also been studied.

Locus. It is generally assumed that microorganisms are the chief agents in soils directly responsible for urea hydrolysis. The reaction may be catalytic in part rather than completely biological. However, soil ureases are partly extracellular being liberated during microbial and plant root metabolism and death. They are also intracellular as part of the soil biomass. In the latter, urease exists as a component of the cytoplasm or is attached to the cell membrane.

In the former case it would be attached to the soil Urease as an endoenzyme will act more rapidly on urea if it enters cells in high concentrations. Similarly, if after lysis, the enzyme is released, it will act more intensely on the substrate.

Ureolysis in soils is primarily due to accumulated urease. Under steady state conditions, 79 to 89% of the urease activity of silty loam soil was due to urease adsorbed on soil colloids that enzymes may change location with time. For example, intracellular urease in a viable cell will be associated with cell debris after subsequent cell death and lysis; this will then be released to aqueous phase as cell membranes are broken down and later be adsorbed to clay surfaces.

Factors affecting urease activity in soil

Optimum pH. The optimum pH of soil urease activity has been reported to be 6.5 to 7.05. The pH optimum of the enzyme is dependent on the buffer used using both phosphate and Tris buffers, found that the pH optimum for a urease active soil extract was 6.5 to 7.0.

Temperature. Urease in soil is more resistant to high temperature than urease in pure preparations and solutions. The inactivation temperature of plant ureases (70°C) are similar to those found in soil.

Numerous studies have shown that urease activity in soils increased with increase in temperature from l0^{o} to 40°C. In some soils, activity has increased very markedly with the increase in temperature from 40° to 70°C; but decreased rapidly above this temperature range. In general, immobilization of enzymes enhances their thermal stabilities. Persistence of immobilized enzymes to thermal denaturation may be related to the stabilization of the tertiary structure of proteins.

Although inactivation of enzymes has been detected between 60^{o} and 70°C urease activity had not been completely destroyed when soils were heated to 75°C for 24hrs or between 80° and 90°C for 48hrs Urease activity in Indian alfisols and vertisols at 100°C was close to zero when a non-buffer method was used for the assay. When soils were heated at 105°C for 24h, urease activity was inactivated completely. This character, inactivation of soil urease at higher temperatures, can be used to minimize N losses following surface application of urea in high urease activity soils.

Moisture content. Even though water plays an important and complex role in the urea hydrolysis, different results on the effect of water content on urease activity have been reported. In some studies urease activity was not appreciably affected by the soil water content. There are reports, however, that urease activity in soil is related either negatively or positively to soil water content.

Urea hydrolysis increases with increasing soil water content up to near field capacity, followed by a decreasing trend thereafter a constant urease activity when the moisture content was increased further beyond field capacity. A higher rate of urea hydrolysis in soils at field capacity than in water logged soils after 24 h incubation. Urea hydrolysis rates decrease below the permanent wilting point and in dry soils.

Reduced ammonia volatilization losses due to the inhibition of urea hydrolysis found at low soil water potentials have been reported. It appears therefore, that the sensitivity of soil urease to lack of soil moisture can be utilized advantageously through fertilizer management.

Urea concentration. As expected the rate of urea hydrolysis in soils treated with small amounts of urea is found to be much slower than that observed with large amounts of urea studies have shown that the rate of hydrolysis of urea by soil urease increases with increase in substrate (urea) concentration until the quantity of added is saturating and its activity becomes constant. The urea concentration between 12.5 to l00 mg N/kg soil was adequate to saturate the urease in soils of the central irrigation areas in South Africa. Urease activity to be positively related to the total in soil. Several other workers have also confirmed that urease activity is independent of N concentration up to 1000 µg N/kg soil; and that responses were similar for different Indian soils tested.

The hydrolysis of urea in soil generally follows Michaelis-Menton kinetics, even though soil is a highly heterogeneous system. This phenomenon, that is the increase in the rate of hydrolysis with the increasing substrate until enzyme is saturated, has been reported by several researchers. However, at very high urea concentrations the hydrolysis rate decreases probably due to either uncompetitive substrate inhibition of the enzyme or denaturation of enzyme at very high concentrations of urea.

The first experiment, with four replicates, was conducted with hydroponics culture in a growth chamber at 25–27°C days with 14 h photoperiod. It consisted of three P and three planting treatments.

The three P treatments were (1) without P addition (P0), (2) 0.25 mmol L^{-1} P as KH_2PO_4, and (3) 0.25 mmol L^{-1} P as phytate-Na. The three planting treatments were (1) four plants of maize (*Zea mays* L.) as a monoculture, (2) eight plants of chickpea (*Cicer arietinum* L. 'Sona') as a monoculture, and (3) two plants of maize and four plants of chickpea as a mixed culture. Seeds of chickpea and maize were surface-sterilized in 5% H_2O_2 for 30 minutes; pregerminated in the dark in a Petri dish with adequate water, and were then planted in quartz sand. After 7–8 d, plants were transplanted to 2 L containers with half-strength nutrient solution for the first 3 d, and thereafter grown in full-strength nutrient solution with or without a supply. The pH of the nutrient solution was adjusted to 6.0 and the solution was renewed every 3 days. During the whole experiment, the containers were continuously aerated.

Oxygen. Oxygen had a significant effect on the rate of hydrolysis of urea added to an Indian soil, oxygen had no effect on the rate of hydrolysis of urea added to Crowley silt loam. Since the urea added to soil is hydrolysed largely, if not entirely, by native soil urease, there is no apparent reason why the activity of urease should be affected by oxygen. Oxygen becomes a limiting factor in urea hydrolysis after found that observed substantial reduction of urease 12h of submergence under flooded conditions of some soils.

Organic matter. Many workers have found that urease activity in soils is positively correlated with organic C and total N, which are indices of organic matter content. Organic matter content of a soil accounted for most of the variations in urease activity. Several workers have observed an increase in soil enzyme activities after incorporation of organic matter into the soil. Further, the constituents of the organic matter also determine the activity of urease in soil.

Soil amendments

Organic manure

Incorporation of organic materials into soil promotes microbial activity and also soil urease activity .The increased of urease activity in the organic levels amended soil has generally been attributed to the increased microbial biomass although additional evidence has shown that plant materials and sludges may directly contribute enzyme to soil. Micro-organisms associated with the organic materials may also contribute to the urease in the soil enzyme the urease activity in soil varies depending upon the type and amount of organic matter added. On addition of decomposed organic matter and farmyard manure (FYM) urease activity increased. However incorporation of undecomposed dried grass had no effect on urease activity. Soil urease activity increased with the addition of FYM, sewage sludge and press mud but not with wheat straw. Urease activity increased significantly with application of organic matter to the soils and the stimulation was greater in salt amended soils.

The activity of soil enzyme may be inhibited by addition of certain organic found that the edition of organic residues (maize and wheat straw) inhibited several soil enzymes. The application of dried sewage sludge and sludge effluents to agricultural lands is becoming a wide- spread practice in the world. The increase observed in urease activity after addition of sludge was attributed to the higher microbial proliferation and activity, while a reduction in urease activity was attributed to the higher concentration of available heavy metals.

Urease activity increased after the first addition of organic matter while subsequent additions failed to sustain high enzyme activity. The increasing urease activity after the addition of organic materials could be due to a trigger molecule or promoter released by the decay of organic amendments that stimulates soil organisms to secrete high level of enzymes. However, less response to subsequent additions may be due to a feedback mechanism that terminates the production of enzymes in a situation where adequate energy sources are available. They also proposed that in a soil receiving constant and regular additions, the process of promotion and suppression may be balanced resulting in a constant level of enzyme activity.

Liming. The addition of amorphous $CaCO_3$, at rates of 2, 4 and 8 per cent decreased urease activity. There observed a negative relationship between natural $CaCO_3$, and soil urease activities. This can be due to inactivation of urease by amorphous $CaCO_3$. It can be due to change in pH upon the addition of $CaCO_3$ or CO_3^{2-} may directly influence the hydrolysis reaction. However, other workers have found that urease activity in soil can be increased by addition of liming.

Herbicides. Certain substituted urea herbicides (Fenuron, Monuron, Linuron and Neburon) inhibit urea hydrolysis by 10 to 30 percent studied the effect. cent in some Italian soil.

None of the herbicides had any significant inhibiting effect on urea hydrolysis even at tenfold. Increase in the concentration of the herbicide.

However, enhanced urease activity in soils treated with glyphosate, paraquat, methanol and carbaryl and an inhibitory effect on immobilized and free urease by methanol, carbaryl and atrazine. Soil urease activity increased with application of monocrotophos, quinalphos, cypermethrin and fenvalerate at 5kg / ha and reduced at higher levels (7.5 and 10kg/h).

Problems associated with high Urease activity. Although urea is considered as equivalent to other nitrogenous fertilizers, poor crop responses to urea have frequently been observed. The rapid hydrolysis of urea due to high urease activity can result in high soil pH values and high ammonium ion concentrations which are conducive to accumulation of ammonia. The major problems observed in urea fertilization are the loss of volatile ammonia gas and ammonia toxicity to germinating seedlings. Also the accumulation of nitrite in the soil following the hydrolysis of urea can result in toxicity and nitrogen losses.

Loss of ammonia and ammonia toxicity. Ammonia toxicity and the loss of N as volatile ammonia are the major problems encountered with fertilizer urea. Equilibrium between ammonium ion and ammonia gas occurs in aqueous solutions of ammonium salts. When enzyme activity is high in soil, the rate of hydrolysis of urea increases, resulting in greater losses of gaseous ammonia. Ammonia volatilization losses from agricultural soils range from 0.4 to 80 per cent of the applied urea nitrogen, and from 3.5 to 24.9 per cent in forest soils depending on the soil conditions and assay conditions. Thus, losses of gaseous ammonia can be sufficiently great to reduce yields 134 and to reduce the efficiency of urea as a nitrogen fertilizer.

Accumulation of nitrite. The alkaline pH and high ammonium ion concentrations resulting from urea hydrolysis in soils do not appreciably affect oxidation of ammonia to nitrite by Nitrosornonas species, but does inhibit the oxidation of nitrite to nitrate by *Nitrobacter* species. In the behaviour of urea, diammonium phosphate and ammonium sulfate in an alkaline soil the highest accumulation of nitrite (260ppm) occurred in the soil to which urea had been added. Toxicity to the plant from nitrite accumulation has been observed subsequent to the addition of urea ferti1izer. Nitrite can be reduced to gaseous N either by biological reactions, or chemical reactions. Those reactions lead to formation of gaseous N which will evolve into the atmosphere, thus, reducing the efficiency of urea.

Effects of cadmium, zinc and lead on soil enzyme activities. The ability of the soil to serve as a habitat for plants, microorganisms and soil-living animals is the most important function of agricultural land. Soil enzyme activity is involved in nutrient cycling and availability to plants and can be used as an index of soil functioning (Nannipieri *et al,* 2003). They are not only essential for plant growth but also equally important for soil fertility. Anthropogenic activities leading to the intentional or unintentional deposition of contaminants may be harmful to soil environment, affect the amount and activities of soil enzymes at different functional levels, reduce the growth and the yield of plants and cause excessive pollutant concentrations in plants.

Heavy metals (HM) are one of the major groups of pollutants in soil environment, arising from repeated applications of sewage sludge, municipal wastes and animal slurries, the activity of smelting industries, impurities in fertilizers and deposition of air pollutants from burning of fossil fuels and various industrial activities. A number of soil characteristics, including biological properties, are profoundly influenced by HM. The strong inhibition of the activities of a variety of enzymes has been reported in metal polluted soils over the past years.

Recent determinations of HM in the sewage irrigation soils in Baoding City of China. The ability of the soil to serve as a habitat for plants, microorganisms and soil-living animals is the most important function of agricultural land. Soil enzyme activity is involved in nutrient cycling and availability to plants and can be used as an index of soil functioning (Nannipieri *et al.,* 2003). They are not only essential for plant growth but also equally important for soil fertility. Anthropogenic activities leading to the intentional or unintentional deposition of contaminants may be harmful to soil environment, affect the amount and activities of soil enzymes at different functional levels, reduce the growth and the yield of plants and cause excessive pollutant concentrations in plants.

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A pot experiment was made with soil after sampling and processing for the pot experiment Five kilogram soil was put in a plastic bucket (20 cm diameter × 20 cm height) after thoroughly mixing with 75g dry poultry manure, 1g urea and 2g di-ammonium phosphate. Cadmium acetate, or zinc acetate or lead acetate was added at five rates in order to gain the regression orthogonal design method which is designed to have 3 factors (three heavy metal elements) and 5 levels (Table 2). In addition, for investigating the combined impact of the three heavy metals, soil was added with the solution containing Cd, Zn and Pb. There were 5 levels for each heavy metal so the total number of treatment is 15 and single element treatments are also 15 (Table 3). Each treatment was replicated three times so the total number of pots in this study was 90.

Table 2: Four soil enzyme activities in the presence of added Cd, Zn and Pb.

Number	Catalase activity[a]		Urease activity[b]		Phosphatase activity[c]		Invertase activity[d]	
1	3.75	(0.09)	0.49	(0.03)	0.71	(0.09)	4.55	(0.40)
2	3.97	(0.05)	0.55	(0.08)	0.64	(0.09)	4.15	(0.12)
3	3.41	(0.08)	0.47	(0.00)	0.96	(0.04)	4.98	(0.34)
4	3.74	(0.02)	0.42	(0.06)	0.69	(0.07)	4.04	(0.30)
5	3.61	(0.03)	0.44	(0.02)	0.45	(0.06)	3.81	(0.35)
6	3.87	(0.09)	0.49	(0.03)	0.44	(0.01)	3.64	(0.21)
7	3.25	(0.01)	0.40	(0.009)	0.55	(0.09)	3.49	(0.40)
8	3.63	(0.09)	0.39	(0.01)	0.45	(0.06)	2.99	(0.38)
9	3.77	(0.07)	0.48	(0.03)	0.82	(0.07)	4.19	(0.50)
10	3.72	(0.04)	0.42	(0.003)	0.59	(0.09)	3.17	(0.03)
11	3.91	(0.04)	0.43	(0.01)	0.39	(0.03)	4.17	(0.19)
12	3.67	(0.001)	0.33	(0.013)	0.59	(0.05)	3.28	(0.17)
13	3.47	(0.03)	0.45	(0.014)	0.79	(0.09)	4.55	(0.19)
14	3.95	(0.09)	0.45	(0.01)	0.67	(0.09)	4.51	(0.28)
15	3.68	(0.06)	0.43	(0.077)	0.68	(0.01)	3.90	(0.14)
Blank test	3.71	(0.01)	0.67	(0.04)	0.55	(0.01)	4.68	(0.39)

Then all pots were added with water to the full soil water holding capacity (WHC) and then equilibrated for 15 days before seeding Canola seeds were sown in each pot after germination, only eight plants with equal size were kept in each pot. Deionized water was added on the soil at 60% of the soil water holding capacity after the thinning and this level was kept during the whole period of the experiment. No symptoms of diseases and pest damage were observed in the period of growth. After growing for 60 d, all plants were harvested and concentrations of Cd, Zn and Pb in shoots were analysed separately. The activities soil enzymes urease and alkaline phosphatase was measured accordingly after the harvest. In this procedure, a solution of urea (10%) and citrate buffer (pH 7) were added to soil in hermetically sealed flasks, and then incubated for 24 h at 37°C. The ammonium content of the centrifuged extracts was determined by a modified indophenol-blue reaction. Controls were prepared without substrate to determine the ammonium produced in the absence of added urea. Soil catalyses activity was measured by potassium permanganate ($KMnO_4$) titration method. In the procedure, a solution of peroxide (0.3%), H_2O_2, was added to soil as substrate.

Effect of added HM in soil on four soil enzyme activities. The effects of the combination of Cd, Zn, Pb and single metal treatments on the four enzyme activities in the soils. The changes of the activities of four enzymes with the added metal concentrations are shown. The catalase activity was inhibited by higher Cd concentrations in the Cd treatment when the added Cd concentration reached 50 mg, whereas the low Cd concentration from 0 to 10 mg activated the enzyme activity.

Table 3: Four soil enzyme activities in the presence of single added Cd, Zn and Pb metals.

HM	Number	Catalase activity[a]	Urease activity[b]	Phosphatase activity[c]	Invertase activity[d]
Cd	1	3.570	0.7236	0.544	5.110
	2	3.556	0.7864	0.462	4.770
	3	3.600	0.6976	0.470	4.599
	4	3.697	0.6831	0.485	4.579
	5	3.508	0.6350	0.388	4.331
Zn	1	3.762	0.6412	0.570	4.162
	2	3.627	0.6363	0.735	4.373
	3	3.584	0.6271	0.821	4.614
	4	3.517	0.6066	0.985	4.416
	5	3.260	0.5346	0.787	3.207
Pb	1	3.803	0.6566	0.560	4.782
	2	3.880	0.7872	0.590	4.805
	3	3.975	0.7930	0.609	4.858
	4	4.063	0.7431	0.640	5.187
	5	4.127	0.7273	0.605	4.642

However, the enzyme activity in the combined Cd, Zn and Pb treatments was decreased by the low Cd concentration whereas it evidently was increased by higher Cd concentrations, and similar trends were also observed with Zn mainly because the enzyme activity was also affected by the interaction of coexisting metals. In the Zn only treatment, the catalase activity decreased with increased Zn concentration from 0 to 800 mg/kg, whereas it increased with the increased Pb concentration in both the Pb alone and multiple-metal treatments. Thus, in all the treatments Pb, either alone or in combination with the other two metals significantly stimulated catalase activity (Fig. 4).

Liu *et al.* (2002) also reported an increase in the enzyme activity in the presence of Pb and showed that the concentration of the exchangeable Pb in soil was very low and the stimulating' effect for the enzyme stemmed from Pb associated with Fe-Mn oxide. Consequently, the observed activation of catalase in the soil in the presence of Pb probably was a result of the reaction between the Pb ion in soil and the functional groups of catalase. At present, very little information is available on the enzyme activation mechanism in the presence of Pb.

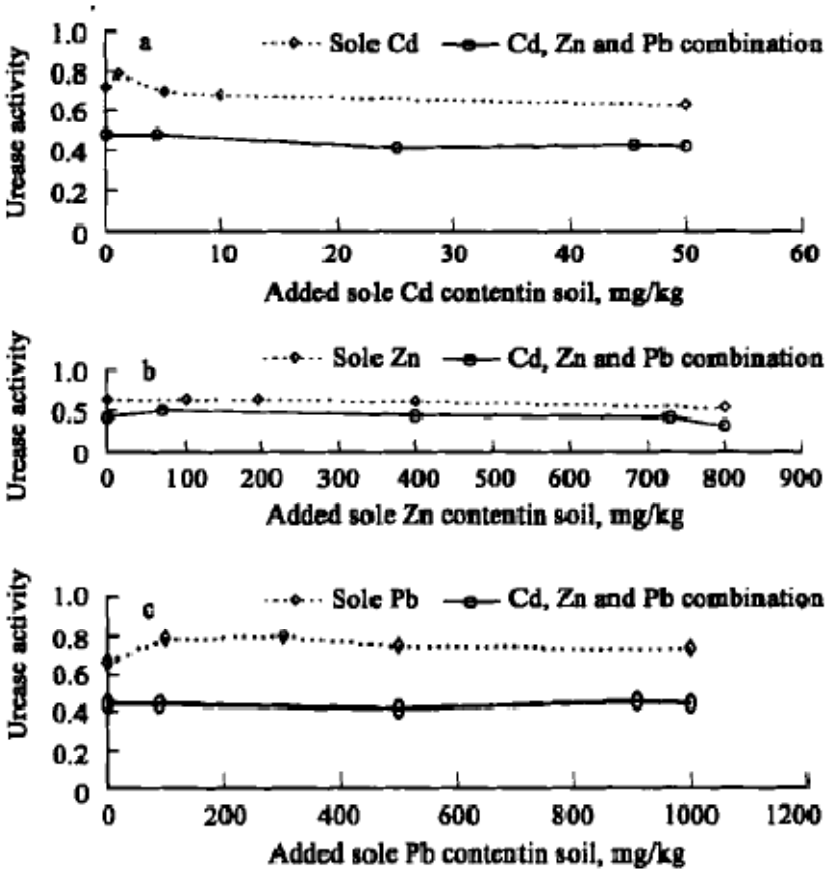

Fig. 4. Comparison of effect of Cd (a), Zn (b), Pb (c) with the combination (Cd+Zn+Pb) in soil on urease activity.

In the study, catalase activity in the soil was lower in the Cd and Zn alone treatments than in the Cd, Zn and Pb combined treatments, whereas it was higher in the Pb alone treatments than in the Cd, Zn and Pb combined treatments (Fig. 1). That is to say, the catalase activity in the soil with Cd was stimulated when Zn and Pb were both added to the soil and the catalase activity in soil with Zn was stimulated when Cd and Pb were both added to the soil. Contrary to what was observed in soil with Cd or Zn, the enzyme activity in soil with Pb was inhibited when Cd and Zn were both added to the soil influence on soil urease activity. Cd and Pb activated urease activity at a low concentration but inhibited this enzyme with rising concentrations in soils when Cd and Pb were the only HM added. Moreover; urease activity was gradually reduced while the concentration of Zn increased in the Zn treatment. In the Cd, Zn and Pb combined treatment, the enzyme activity was inhibited with the increase of Cd and Zn concentration, but the effect of Pb on the enzyme was not evident with the increase of its concentration.

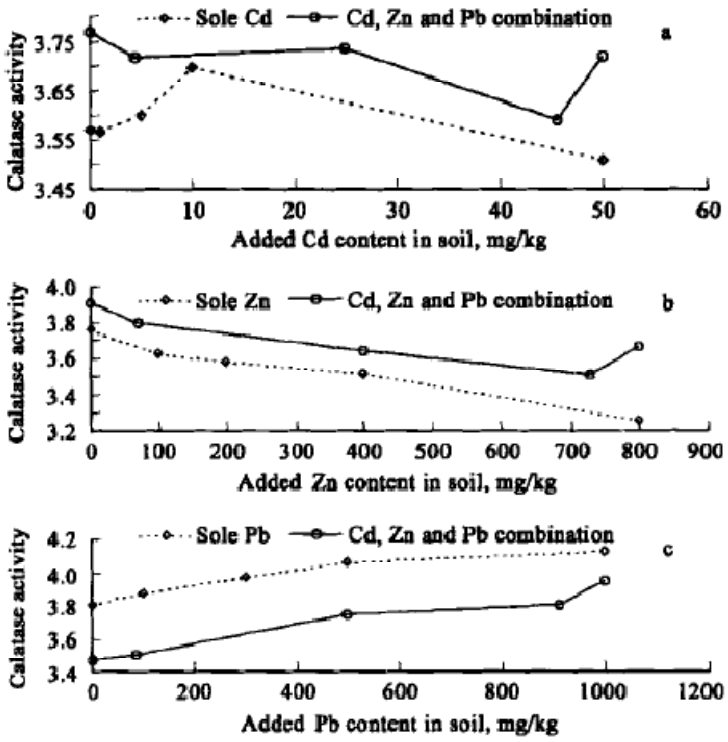

Fig. 5. Comparison of effect of Cd (a), Zn (b), Pb (c) with the combination (Cd+Zn+Pb) in soil on catalase activity.

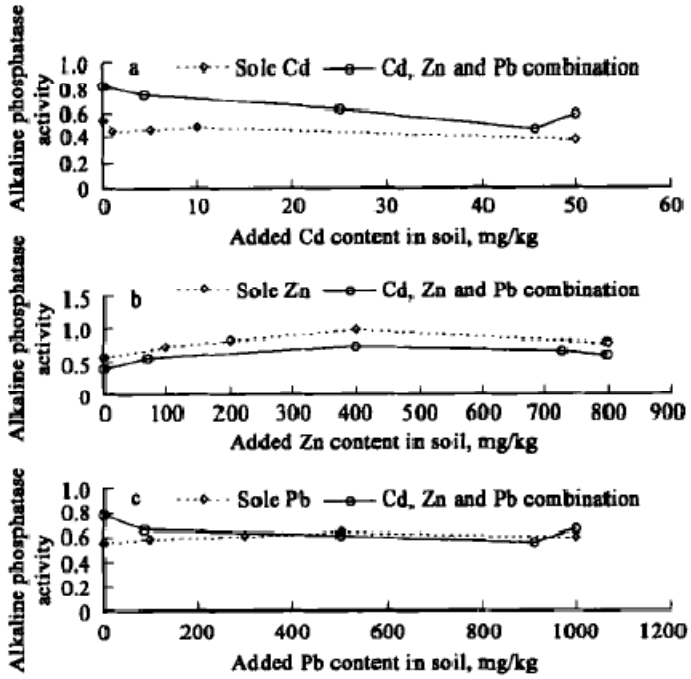

Fig. 6. Comparison of effect of Cd (a), Zn (b), Pb (c) with the combination (Cd+Zn+Pb) in soil on alkaline phosphatase activity (Anetta *et al,* 2013).

Therefore, Cd, Zn and Pb combination, especially Pb, has a protective effect on soils contaminated with a combination of Cd, Zn and Pb than in soils amended with Cd, Zn and Pb separately. Urease activity was more inhibited by HM combination treatments than single Cd, Zn and Pb treatments, being reduced by 20-40%.

Therefore, multiple-metal treatments inhibited urease activity far more than the single HM treatments, with the synergistic inhibiting effect of Cd and Zn being particularly obvious To sum up, each of the four soil enzyme activities studied here was affected differently by the added Cd, Zn and Pb combinations or single metal treatments. The degree of enzyme inhibition or activation varied with the concentration and different HM ion and the type of enzyme assayed. The inhibition or activation of these enzyme activities in soils in the presence of HM combination was a result of the interaction of HM and the reaction between the HM in solution and the functional groups of enzymes (Kulkarni and Kale, 2014).

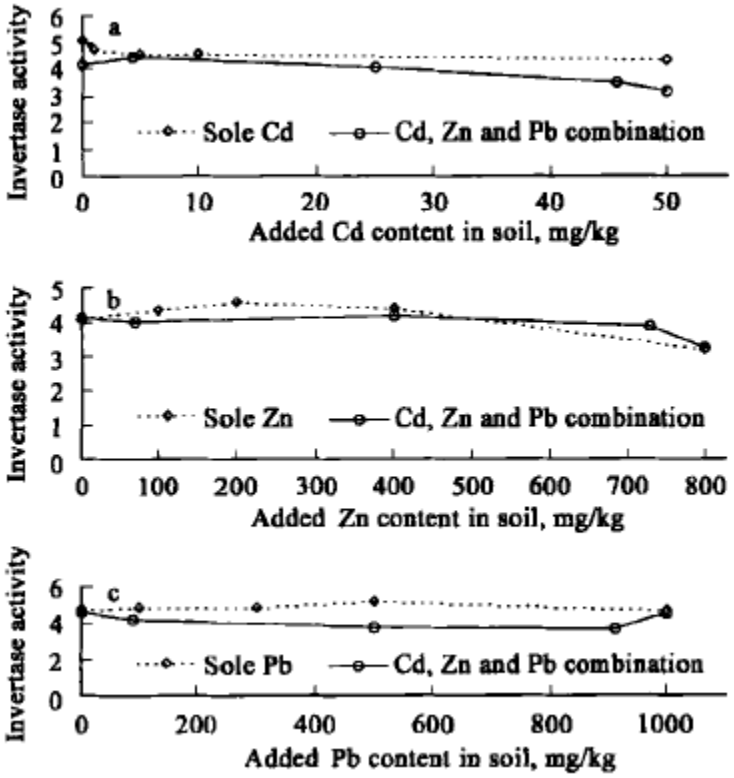

Fig. 7. Comparison of effect of Cd (a), Zn (b), Pb (c) with the combination (Cd+Zn+Pb) in soil on invertase activity.

The chemical and physical properties of the soil, such as organic matter content, type and amount of clay and soil pH, influence the toxic effect of trace elements. These processes are perhaps responsible for the variation in the inhibition or activation observed in soil with different heavy metal as reported by (Kumar *et al.,* 2000).

CONCLUSION

Enzymes respond to soil management changes long before other soil quality indicator changes are detectable. Soil enzymes play an important role in organic matter decomposition and nutrient cycling. Some enzymes only facilitate the breakdown of organic matter (e.g., hydrolase, glucosidase), while others are involved in nutrient mineralization (e.g., amylase, urease, phosphatase, sulfates). With the exception of phosphatase activity, there is no strong evidence that directly relates enzyme activity to nutrient availability or crop production.

The relationship may be indirect considering nutrient mineralization to plant available forms is accomplished with the contribution of enzyme activity.

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