

Effect of Physico-Chemical Properties on Soil Enzyme Urease Activity in Some Soils of Ranga Reddy District of Telangana State, India

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ABSTRACT

To study the distribution of soil enzyme urease in some of the vegetables growing soils of Ranga Reddy District of Telangana State, as Ranga Reddy district is near to metropolitan city Hyderabad and most of the farmers are involved in growing vegetables and are supplying to the nearby city to various vegetable markets present nearby. Soil enzyme activity is influenced by physico-chemical properties on soil enzyme. The soil was analysed for the physicochemical properties and they were correlated with the urease enzyme activity in the soils. The soil samples were analysed for the physicochemical properties like pH, EC, available nutrients, texture and organic carbon and soil enzyme activity was assayed. The pH ranged from 5.7 to 8.9, electrical conductivity from 0.1 to 1.23 dSm⁻¹ and organic carbon from 0.13 to 1.48 %. The available Nitrogen varied from 201.5 to 472.5 kg ha⁻¹. The available P₂O₅ status in the soils varied from 11.6 to 79.1 kg ha⁻¹. The range of available K₂O ranged from 118 to 411 kg ha⁻¹. The urease activity of surface soils expressed as µg of NH₄⁺ released g⁻¹ soil h⁻¹. The ranges of urease obtained in the present instigation from 5.9 to 16.0 with an average value of 8.74. Correlation between soil properties and soil urease were calculated and these results showed that urease activity was positively significantly correlated (r = 0.943**) and available nitrogen (r = 0.510**), while no significant correlation was observed with clay, silt and pH.

Keywords

Physico-chemical, Urease, Organic carbon, Electrical conductivity.

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Introduction

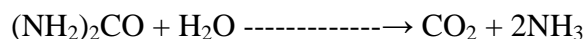
Soil enzymes play an important role in mineralization of nitrogen, phosphorus and sulfur. Mineralization is the process of transformation of organically bound elements into mineral from which is readily taken up by plants and is crucial to plant nutrition. The process of mineralization is brought about by soil microorganisms and also by the abiotic enzymes present in soil. They play an important role in catalyzing several important

reactions necessary for the life processes of microorganisms in soils and their by stabilizing soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling (Dick *et al.*, 1994). These enzymes play an important role in agriculture and particularly in nutrient cycling (Tabatabai, 1994; Dick, 1997). In this regard, all soils contain a group of enzymes that determine soil metabolic processes (McLaren,

1975) 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. Soil enzyme activities have potential to provide unique interactive biological assessments of soils because of their relationship to soil biology, ease of measurement and rapid response to change in soil management (Dora *et al.*, 2008).

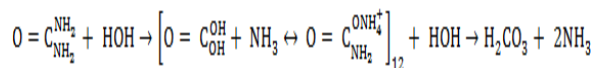
Among the different facets of the subject of soil enzymes, the *in situ* behavior of these enzymes in heterogeneous environment of the soil system in respect of their thermal sensitivity, pH effects, kinetic parameters, activation and inhibition by different soil amendments are of prime importance. The present investigation was specially designed for studying the physico-chemical behavior of enzymes in the soil, their distribution and activity in some limited ecosystems with special reference to their kinetics and related activation parameters. Urease (urea amidohydrolase, EC 3.5.1.5) is the soil enzyme that catalysis the hydrolysis of urea in soil to CO₂ and NH₃:

Urease



It belongs to amidases group of enzymes that also includes glutaminase and asparaginase and acts on C-N bonds other than peptide bonds in linear amides. Since two C-N bonds are present in urea, which are broken by the process of hydrolysis of urea by urease, due to the presence of the stoichiometric relation in the equation which is the result of component reactions. To determine the mechanism of urease action, a number of studies have been conducted and the work by Blakeley *et al.*,

(1969) has provided convincing evidence that carbamate is the intermediate in a two-step reaction. The reaction is summarized by Reithel (1971) as follows:



Urease was the first enzyme protein to be crystallized by Sumner in 1951. Urease is very widely distributed in nature. It has been detected in microorganisms, plants and animals. Its presence in soil was first reported by Rotini, (1935). The basic information about the enzyme urease in soil system was provided from the studies of Conrad, (1940, 1942 and 1943).

Materials and Methods

Forty soil samples were collected from different mandals of Ranga Reddy district of southern Telangana zone. These samples were air dried and passed through 2 mm sieve before use. These soils samples were analyzed for their different soil properties viz. physical, physico-chemical and chemical properties by using standard procedures (Jackson, 1973). Soil pH-The pH of soil was determined in 1:2.5 soil- water ratio as described by Jackson (1973) using a digital combined glass electrode pH meter (model DI-707). Electrical Conductivity (dSm⁻¹) – The EC was determined in 1:2.5 ratio of soil to water extract as detailed by Jackson (1973) using a digital conductivity bridge and expressed in dSm⁻¹ (model DI – 909). Organic Carbon (%) Organic carbon in soil was estimated by Walkley and Black (1934) method and as described by Jackson (1973). It is expressed as percentage of organic carbon. Mechanical Analysis-Mechanical composition of soils was determined by Bouyoucos hydrometer method (Bouyoucos, 1962). The relative proportion of sand, silt and clay of soils were determined to describe their textural classes were carried out with the help of international

triangle (Singh, 1980). Available Nitrogen (kg ha^{-1}) -The available nitrogen was determined by Macrokjeldhal distillation method using alkaline potassium permanganate method as described by Subbaiah and Asija, (1956) and modified alkaline KMnO_4 method by Sahrawat and Barford and expressed as kg ha^{-1} . Available Phosphorus (kg ha^{-1}) – The available phosphorus was determined by Olsen's method (1954). The blue colour was developed by using L-ascorbic acid in this method. The intensity of blue colour developed was measured by using spectrophotometer at 420 nm and expressed as kg ha^{-1} . Available Potassium (kg ha^{-1}) – The available Potassium in soil was estimated by using neutral normal ammonium acetate extractant (Jackson, 1967) by using Elico flame photometer and expressed as kg ha^{-1} .

Urease activity was assayed by quantifying the rate of release of NH_4^+ from the hydrolysis of Urea as described by Tabatabai and Bremner (1972), but with some modifications as suggested by Dorich and Nelson (1983) and Rao (1989). Urea solution (0.2 M): This was obtained by dissolving 1.2 g of Urea in 80 ml distilled water and volume was made up to 100 ml. Potassium chloride (2 M) - Silver Sulphate (100 ppm) KCl- Ag_2SO_4 solution: 100 mg of Ag_2SO_4 was dissolved in 700 ml of distilled water to which 300 ml of water containing 149 g of KCl was added. MgO: Magnesium oxide was heated in an electrical furnace at 500°C for an hour and the powder was collected in dessicator and stored in a tightly stoppered bottle. 4% Boric acid: 40 g of Boric acid was dissolved in a beaker containing hot distilled water about 800 ml. Then 5 ml bromocresol green and 15 ml of methyl red were added and the volume was made upto 1 litre with hot distilled water. 0.005 N H_2SO_4 : This solution was prepared by taking 5 ml of 1N H_2SO_4 is taken in a 1 litre volumetric flask and make up to the mark by the addition of distilled water. Soil sample (5

g) was taken in 25 x 150 mm capacity screw capped tubes. 9ml of THAM buffer was added, the contents were gently mixed followed by addition of 1ml of 0.2M urea. The contents were then swirled and incubated at $37 \pm 0.5^\circ\text{C}$ for two hours in BOD incubator. The reaction was terminated by addition of 50ml of KCl- Ag_2SO_4 solution. The contents were agitated on mechanical shaker for one hour to release all NH_4^+ formed and the suspension was allowed to settle. Thirty ml of the supernatant with KCl- Ag_2SO_4 extract was taken and transferred to Kjeldahl flask. To this a pinch of MgO was added which was kept at one end of the distillation unit. During steam distillation for 4 min, the solution containing MgO was heated. The ammonia was released into boric acid containing mixed indicator through a tube dipped in the solution. The ammonia released would change the color of the solution from pink to pale green at the end of the distillation. This was titrated against standardized 0.005N H_2SO_4 and the amount released was calculated and expressed as μg of NH_4^+ released g^{-1} soil h^{-1} .

Results and Discussion

The urease activity of surface soils expressed as μg of NH_4^+ released g^{-1} soil h^{-1} . The ranges of urease obtained in the present instigation from 5.9 to 16.0 with an average value of 8.74 are similar to those obtained by Srinivas (1991), Yadav and Tarafdar (2001), Ndakidemi *et al.*, (2006), Tripathi (2006), Chaudhuri, (2009), Reddy, (2008) and Vandana (2012) from India and various parts of the world. The correlation between soil properties and soil urease was calculated and these results showed that urease activity was positively significantly correlated ($r = 0.943^{**}$) and available nitrogen ($r = 0.510^{**}$), while no significant correlation was observed with clay, silt and pH (Fig. 1). This is in conformity with the findings from other

studies also (Chhonkar and Tarafdar, 1994; Hu and Cao, 2007; Shilpashree and Kotur, 2009 and Vandana, 2012).

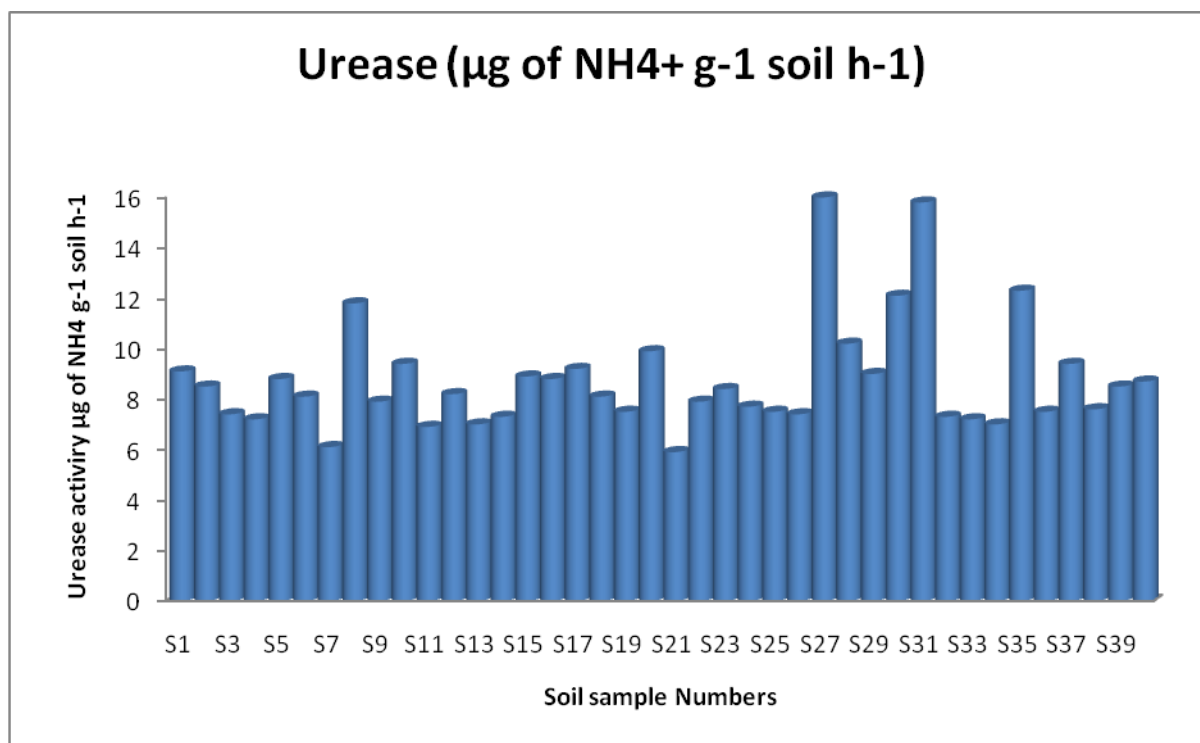
The differences in the level of soil urease activity are caused primarily because of soil type, depending on origin and development conditions and its content of organic matter. Hence each type of soil has its own inherent level of enzyme activity (Vandana, 2012). In addition the soil organic carbon and mineral particles may also adsorb enzymes and protect enzymes against microbial degradation (Zhang *et al.*, 2010). Soil enzymes appear to be immobilized on soil organic matter and the soil organic matter may be indexed by both organic carbon and total nitrogen. Hence significant positive correlation between urease activity with the total and available nitrogen is anticipated Shilpashree and Kotur, (2009) showed a positive correlation between urease activity and organic carbon. The variation in urease

activity is essentially accounted by organic carbon content, since the organic constituents contribute significantly to protection of native enzymes.

Several properties like organic carbon, total nitrogen, soil pH, clay content moisture content have been shown to influence enzyme activity. 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 biological processes.

Enzyme activities of soils are usually correlated either with their organic C and/or total N contents. Soil enzymes activities are usually significantly correlated to soil pH (Gianfred *et al.*, 2005). A strong correlation between the enzyme activity and total number of microorganisms was determined by Cristian and Aurelia (2010).

Fig.1 Soil enzyme urease activity in various samples



The studies on effect of soil properties on the levels of soil urease activity have showed that urease activity tends to increase with the content of organic matter and clay. Non-calcareous soils and heavy textured soils tend to have higher activity than calcareous soils (Skujins and McLaren, 1969). Furthermore, it has been found that saline and clay soils tend to have lower urease activity and soils under dense vegetation tend to have higher urease activity (Myers and McGarity, 1968). Gould *et al.*, (1973) and Tabatabai, (1977) reported that in the soil profiles, the activity of urease enzyme decreased with depth. Zantua *et al.*, (1977) reported that soil urease activity was largely influenced by the organic matter content, pH and specific soil area than any other soil properties and also reported that the soil urease activity is highly significant with total nitrogen.

Relationship between, organic matter content and soil urease activity have been reported by many workers Gould *et al.*, (1973) and Tabatabai, (1977). However, Pancholy and Rice, (1973) observed that urease activity in nine Oklahoma surface soils was influenced by the type of vegetation. Since, it was not significantly correlated with organic carbon or pH. Dalal, (1975) reported that organic carbon and cation exchange capacity were positively correlated with urease activity in soils and also reported that urease activity was significantly correlated with oxalate extractable amorphous iron or aluminum, but not with pH and clay. Zantua *et al.*, (1977) found that urease activity was significantly correlated with organic carbon, total nitrogen and CEC. Speir *et al.*, (1980) found that urease activity was significantly correlated with total nitrogen, total sulphur, organic carbon and protease activity in soil, while Dash *et al.*, (1981) reported a positive correlation between urease activity, total nitrogen, organic carbon, silt + clay and specific conductance and a negative correlation with pH and moisture. Sahrawat,

(1984) found that urease activity in wetland dry soils was significantly correlated with total nitrogen and organic carbon, but not significantly correlated with CEC, clay, pH, active iron or active manganese and concluded that organic matter content measured by organic carbon and total nitrogen accounted for most of the variations in urease activity. Hussain *et al.*, (1990) reported that organic matter content of the soil is the main factor controlling variation in urease activity and found significant positive correlation of urease activity with total nitrogen, organic carbon and no significant correlation with clay content, sand and silt content. The activities of total urease (intra- and extracellular; after chloroform fumigation without toluene treatment), intracellular urease (activity of the microbial biomass) and extracellular urease (before chloroform fumigation without toluene treatment) that is, (total activity minus extracellular activity) were significantly correlated with microbial biomass carbon (Klose and Tabatabai, 1999).

Simple correlation study was carried out between soil properties and enzymes activity and it showed that urease had a significant positive correlation with organic carbon content. The higher correlation between the enzyme activity and organic carbon content is because the latter is the seat of microbial population and activity. It was also observed that higher dehydrogenase activity in surface soil (0-10 cm depth), having higher organic matter content (Shilpashree and Kotur, 2009).

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