**Effect of Temperature on Alkaline Phosphatase Activity in Alfisols and Vertisols of**

**Andhra Pradesh**

**D.Srinivas1 and P.Chandrasekhar Rao2**

**1Associate Dean, Agricultural College, Naira (ANGRAU) and**

**2Dean of Agriculture (Retd), Hyderabad(PJTSAU)**

**A B S T R A C T**

Soil enzymes play an important role in catalyzing several important reactions necessary for the life processes of microorganisms in soils thereby stabilizing the soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling. Alkaline phosphatase belongs to the group of Phosphomoesterases and helps in the mineralization of phosphorus from organic form. The activity of enzymes in soils is influenced by the temperature, moisture and pH of soil. When the temperatures are increased due to various changes caused by global warming they have a profound influence on soil enzymes. Every enzyme has its optimum temperature below which the enzyme activity is less due to inactivation**.** Further, with an increase in temperature, the enzymes get denatured resulting in a decrease in nutrient availability and indirectly affecting productivity. To study the effect of temperature on soil enzyme activity, four alfisols and four vertisols were collected and laboratory incubation studies were carried out at different temperatures ranging from 20oC to 90oC. The alkaline phosphatase activity (µg of 4-nitrophenol g-1 soil h-1) ranged from 23.08 to 120.55 in alfisols while in vertisols the activity varied from 66.58 to 536.88. Temperature coefficient values (Q10) were calculated in the temperature range of 20 to 90oC. These values depend on the type of soil which varied from 0.31 to 1.88 in alfisols and 0.38 to 1.92 in vertisols.

**Key words**: Alfisol, Alkaline phosphatase, Temperature, Vertisol, Temperature quotient

**Introduction**

The abiotic enzymes present in the soil play an important role in catalyzing several important reactions necessary for the life processes of microorganisms in soils thereby stabilizing soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling. When the temperature is increased due to various changes caused by global warming and other aspects, it has a profound influence on soil enzymes and indirectly on agricultural productivity. Agriculture is influenced by climate change, the temperature being one of the key components Phosphatase is the important enzyme and its activity plays a fundamental role in the transformation of P from soil organic matter into available forms. Phosphatase enzymes are produced by bacteria, fungi and plant roots and serve to cleave a phosphate group from its substrates, transforming complex. The rhizosphere is a narrow region of the soil that is directly influenced by root and mycorrhiza secretions of phosphatase and other enzymes thus sustaining dense populations of root-associated and free-living microorganisms (**Srinivas *et al****.,*2000) Therefore, soil contains large quantities of intracellular (in living microbial cells) and extracellular (secretions of living cells or dead cellular material) phosphatases. Phosphatases can furthermore be stabilized in the soil on surface-reactive particles (e.g. clay and iron or aluminum oxides). This geochemically immobilized and yet enzymatically active fraction accounts for the enzymatic activity exhibited by soil, even in the absence of living organisms. These enzymes play key roles in the overall process of organic matter decomposition and organic nitrogen in soil systems which are important reactions necessary for the live processes of microorganisms in soils and stabilization of soil structure, decomposition of organic waste, organic matter formation and nutrient cycling (**Dick *et al****.,* 1994). During the decomposition of organic matter, these enzymes are constantly synthesized, accumulated, inactivated and decomposed in soils, hence they play an important role in Agriculture (**Tabatabai** 1994, **Dick**, 1997 and **Vandana** 2012). Soil enzymes have the 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). Phosphorus is present in the soil in several organic and inorganic forms, and only a small fraction of Porg is susceptible to releasing available phosphate after a phosphatase reaction (**Johnson** *et al* 2003). Hence the present investigation was undertaken to study the effect of temperature on soil enzyme alkaline phosphatase activity in alfisols and vertisols of Andhra Pradesh.

# Material and Methods

The procedure of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) were adopted for the assay of alkaline phosphatase activity in soils. Four alfisols and four vertisols soil samples were taken for the study

Modified Universal Buffer (MUB) Stock: The stock of MUB was prepared by mixing 12.1 g of Tris (hydroxymethyl) aminomethane (THAM), 11.6 g of maleic acid, 14 g of citric acid and 6.3 g of boric acid in 488 ml of 1N sodium hydroxide and the solution was diluted with weather and by their experience choose highly adaptive varieties to the local climate and in the soils of arid and semi-arid tropics, the soil available nitrogen is grossly inadequate for sustainable agriculture unless it to 1 litre with distilled water. Modified Universal Buffer (pH 6.5): 200 ml of MUB stock was transferred to 1 litre beaker and kept on a magnetic stirrer and the pH of the solution was adjusted to 6.5 with 0.1N HCl and volume was made up to 1 litre with distilled water.

Modified Universal Buffer (pH 11): 200 ml of MUB stock was transferred to 1 litre beaker and kept on a magnetic stirrer and the pH of the solution was adjusted to 11 with 0.1N NaOH and volume was made up to 1 litre with distilled water. The MUB buffer was wrapped with carbon paper and stored in a refrigerator.

P-nitrophenyl phosphate solution (0.025M): This was prepared by dissolving 0.420 g of the disodium salt of p-nitrophenyl phosphate in 40ml of MUB pH 11 (for assay of alkaline phosphatase) and the solution was diluted to 50 ml with MUB of the same pH. The solution was wrapped with carbon paper and stored in a refrigerator.

Calcium chloride (0.5M): This was prepared by dissolving 73.5g of CaCl2.2H2O in distilled water and made up to 1 litre.

Sodium hydroxide (0.5M): 20 g of sodium hydroxide was dissolved in 700 ml of distilled water and diluted to 1 litre with water.

Standard p-nitrophenol solution: Primary stock solution of 1000 µg ml-1 of p- nitrophenol was prepared by dissolving 1 g of p-nitrophenol in distilled water and made up to 1 litre. From this, secondary stock of 100 µg ml-1 and 20 µg ml-1 solutions were prepared. Working standards of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg ml-1 were prepared from 20 µg ml-1 stock and the absorbance of these standards were recorded at 420nm in spectrophotometer. This was used for the standard curve.

# Procedure

To 1 g of soil sample taken in glass tubes, 4 ml of modified universal buffer pH 6.5 (for assay of acid phosphatase) was added followed by addition of 1 ml of 4-nitrophenyl phosphate solution. The glass tubes were swirled for few seconds to mix the contents, stoppered and incubated for one hour at 37 ± 0.5oC in BOD incubator. To these, 1 ml of 0.5M CaCl2 was added followed by addition of 4 ml of 0.5M NaOH to deactivate the enzyme and to extract the 4-nitrophenol liberated. The glass tubes were swirled and the soil suspension was filtered through Whatman No. 42 filter paper. The absorbance of the yellow color of 4-nitrophenol liberated due to hydrolysis of the substrate by phosphomonoesterases was measured at 420 nm. Controls were run simultaneously following the same procedure except adding 1 ml of 4-nitrophenyl phosphate after the addition of 1 ml of 0.5M CaCl2 and 4 ml of 0.5M NaOH. Corrections were made for control / blank values

# Results and Discussion

The results regarding to the effect of temperature on soil alkaline phosphatase activity in alfisols and vertisols are depicted graphically in Figure1 and 2. Alkaline phosphatases activity of all soils used in the study increased with increase in temperature from 20 – 60oC and thereafter activity decreased slowly till 70oC and rapidly decreased with further increase in temperature to 90oC. Denaturation occurred beyond 60 oC in alfisols and vertisols

The average alkaline phosphatase activity in alfisols varied from 23.08 to 120.55 µg of 4-nitrophenol g-1 soil h-1 with the increased temperature from 20-60oC and thereafter the activity decreased to 78.14 µg of 4-nitrophenol g-1 soil h-1 at 70 oC and further decreased to 21.48 µg of 4-nitrophenol g-1 soil h-1 at 90 oC. Among the alfisols, A3 recorded higher activity of 210.60 µg of 4-nitrophenol g-1 soil h-1 followed by A3 (158.80) and A2 (98.25) and A1 (14.85). Similarly, in vertisols, the average acid phosphatase activity varied from 66.58 to 536.88 µg of 4-nitrophenol g-1 soil h-1 with the increased temperature from 20-60oC and there after the activity decreased to 367.74 µg of 4-nitrophenol g-1 soil h-1 at 70 oC and further increased to 80.98 µg of 4-nitrophenol g-1 soil h-1 at 90 oC. Among the vertisols, V3 recorded higher activity of 741.30 µg of 4-nitrophenol g-1 soil h-1 followed by V4 (688.60), V1 (377.00) and V2 (340.60). In both the soils, the acid phosphatase activity beyond optimum temperature of 60oC was decreased due to loss of thermal stability of enzyme.

**Fig.1 Effect of temperature on soil acid phosphatase activity in alfisols**

**Fig.2 Effect of temperature on soil acid phosphatase activity in vertisols**

The temperature coefficient values (Q10) were calculated in the temperature range of 20 to 90oC for the alfisols and vertisols and are presented in tables 1 and 2 , respectively. These values depend on the type of soil and it was observed the Q10 values were found higher in vertisols compared to alfisols. The values varied from 0.48 to 1.88 in A1, 0.31 to 1.81 in A2, 0.54 to 1.67 in A3 and 0.34 to 1.55 in A4 while in vertisols, the Q10 values varied from to 0.48 to 1.90 in V1, 0.41 to 1.90 in V2, 0.37 to 1.92 in V3 and 0.43 to 1.87 in V4.

**Table 1 : Temperature Coefficient Values (Q10) of Alkaline phosphatase in alfisols**

|  |  |
| --- | --- |
| **Temperature range****(oC)** | **Temperature Coefficient Values (Q10) of** **Acid phosphatase in Alfisols** |
| **A1** | **A2** | **A3** | **A4** |
| 20-30 | 1.88 | 1.81 | 1.67 | 1.55 |
| 30-40 | 1.66 | 1.79 | 1.41 | 1.55 |
| 40-50 | 1.56 | 1.71 | 1.35 | 1.55 |
| 50-60 | 1.18 | 1.63 | 1.30 | 1.55 |
| 60-70 | 1.24 | 0.80 | 0.67 | 0.48 |
| 70-80 | 1.45 | 0.55 | 0.64 | 0.48 |
| 80-90 | 0.45 | 0.31 | 0.54 | 0.34 |

**Table 2 : Temperature Coefficient Values (Q10) of Acid phosphatase in Vertisols**

|  |  |
| --- | --- |
| **Temperature range****(oC)** | **Temperature Coefficient Values (Q10) of acid phosphatase in Vertisols** |
| **V1** | **V2** | **V3** | **V4** |
| 20-30 | 1.90 | 1.90 | 1.92 | 1.87 |
| 30-40 | 1.86 | 1.86 | 1.71 | 1.74 |
| 40-50 | 1.82 | 1.60 | 1.55 | 1.59 |
| 50-60 | 1.81 | 1.54 | 1.51 | 1.35 |
| 60-70 | 0.73 | 0.74 | 0.71 | 0.61 |
| 70-80 | 0.50 | 0.66 | 0.49 | 0.53 |
| 80-90 | 0.48 | 0.41 | 0.37 | 0.43 |

Temperature has a profound effect and controls soil enzyme activities, changing enzyme kinetics and stability, substrate affinity and enzyme production because it can influence the size and activity of microbial biomass. Alkaline phosphatase activity of soils increased with temperature from 20oC to 60oC and decreased constantly with further increase in temperature to 90oC (Rao, 1989 and Vandana, 2012). Similarly, Fauvel and Rouquerol (1970) reported maximum alkaline phosphatase activity at 60oC which later rapidly decreaed upto 100oC. The temperature dependence of soil hydrolase activities was described by Arrhenius equation (Cepeda *et al.,* 2007). They measured the Q10 of nine different enzymes in three different soils and found that the Q10 at 20oC exceeded 2.0 only for B-glucosidase in one of the soils.

Energy of activation (Ea) of alkaline phosphatase was calculated by the least square analysis in alfisols and vertisols and are varied from 31.7 $\pm $ 1.1 (A4) to 68.3 ± 3.2 (A4). Similarly, the Ea values in vertisols varied from 28.6 ± 0.6 (V4) to 51.7±1.2 (V2). The results in accordance to the finds of Khaziev (1975). Similary, Dick and Tabatabi (1989) reported that the variation in the energy of activation parameters was attributed to the heterogeneity in the composition and state of soil enzymes.

The activity of any chemical reaction increases with temperature, for every 100C rise in temperature the rate of the reaction approximately increase by two folds. The rate of enzyme catalyzed reaction increases as the temperature increases until optimum temperature is reached above which the rate begins to decrease because of denaturation of enzyme. The same pattern has been observed in soil enzymes by a number of investigators except the fact that the temperature over which the soil enzymes retain their stability is much higher than that for the free enzymes. This is attributed to the stability effect due to the immobilization of the soil enzymes on soil particulate matter. Activation energies are parameters that mechanistically link enzyme kinetics and temperature responses through the Arrhenius function. Enzyme catalyzed reactions generally show lower activation energies than uncatalyzed reactions, so the temperature sensitivity of the abiotic reactions may be higher (Tabatabai, 1982). Several studies have demonstrated that the temperature sensitivity of extracellular enzymes changes seasonally (Fenner *et al.,* 2005; Koch *et al.,* 2007; Trasar-Cepeda *et al.,* 1988 and Wallenstein *et al.,* 2009). It is known that the temperature needed to deactivate enzymes in soils is about 10 oC higher than the temperature needed to inactivate the same enzyme in absence of soil. This has been generally attributed to the immobilization of soil enzymes on soil colloids and cell debris (Tabatabai, 1982; Srinivas, 1993; Raman and Reddy, 1998; Srinivas and Raman, 2000 and Vandana 2012). Changes in temperature not only effect the enzyme production but also effect enzyme degradation rates in the environments. Increase in temperature results in enzyme production rates of alkaline phosphatase with shifts in microbial population and influence the biogeochemical cycles in the soil.

**Acknowledgements**

The authors are thankful to Acharya N.G.Ranga Agricultural University for providing necessary financial support in conducting the experiment.

# References

Battisti D. S., Naylor R. L. 2009 Historical warnings of future food insecurity with unprecedented seasonal heat. Science 323, 240–244. (doi:10.1126/science.1164363).

Cepeda, M.C.T and Sotres, F.G. 1988. Kinetics of acid phosphatase activity in various soils of Galicia. *Soil Biology and Biochemistry.* 20(3): 275 -280

Dick, R.P. 1994. Soil enzyme activities as indicator of soil quality. In J.V. Doran,

D.C. Coleman, D.F. Bezdicek and V.A. Stewart (eds.) – *Defining Soil Quality for Sustainable Environment*, Soil Science Society of America, American Society of Agriculture, Madison. 107 – 124.

Dick, R.P. 1997. Soil enzyme activities as integrative indicators of soil health. In C.E. Pankhurst, B.M. Doube and V.V.S.R. Gupta (eds.) – *Biological Indicators of Soil Health*, CAB International, Wellingford. 121 – 156

Dora, A.S., Domuta, C., Ciobanu, C and Sandor, M. 2008. Field management effects on soil enzyme activities. *Romanian Agricultural Research*. 25: 61-68.

Eivazi, F and Tabatabai, M.A. 1977. Phosphatases in soils. *Soil Biology and Biochemistry*. 9: 167-172.

Fauvel, S. and Rouquerol, T. 1970. The phosphatase test considered as an index of soil activity and evolution. In Soil enzymes (ed R G Burns), Academic Press inc, New York.

Fenner N, Freeman C, Reynolds B 2005 Observations of a seasonally shifting thermal optimum in peatland carbon- cycling processes; implications for the global carbon cycle and soil enzyme methodologies. *Soil Biology and Biochemistry* 37:1814–1821

Khariev, F. Kh. 1975. Thermodynamic characteristics of enzyme reactions in soil. Biol Nauki 10: 121-127. Chemistry abstracts 42394, 1976.

Koch, O., Tscherko, D., Kandeler, E., 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil EAs in organic alpine soils. *Global Biogeochemistry Cycles* 21, 1–11.

Porter J. R., Semenov M. A. 2005Crop responses to climatic variation. Phil. Trans. R. Soc. B 360, 2021–2035]

Raman S and Reddy M.S.R.L 1998 kinetics and activation of L-asparaginase in Alfisols and Vertisols. *Journal of Indian Society of Soil science* 46 (3) 367-370

Rao, S.V. 1989. Distribution, Kinetics and some interactions of urease and phosphatases in soils*. Ph.D. Thesis*. Acharya N G Ranga Agricultural University, Hyderabad.

Srinivas, D. 1993. Phosphomonoesterase activity in some soils of Andhra Pradesh. Distribution, kinetics and variation due to plant cover, pesticide application and submergence. *Ph.D. Thesis.* Acharya N G Ranga Agricultural University, Hyderabad.

Srinivas, D., Raman, S and Rao, P. C. 2000. Influence of plant cover on acid and alkaline phosphatase activity in two soils of Andhra Pradesh. *The Journal of Research,* ANGRAU. 28(4): 40-47.

Tabatabai, M.A and Bremner, J.M. 1969. Use of P-nitrophenyl phosphate for assay of soil phosphatase activity*. Soil Biology and Biochemistry.* 1: 301-307

Tabatabai, M.A. 1982. In A.L. Page (eds.) – *Methods of Soil Analysis, Part 2, Soil Enzymes,* American Society of Agronomy Madison. 903 – 948.

Tabatabai, M.A. 1994. Microbiological and biochemical properties. In R.W. Weaver, J.S. Angle and P.S. Bottomley (eds.) – *Methods of Soil Analysis, Part 2, Soil Enzymes,* Soil Science Society, Society of America Madison. 775 – 833

Trasar-Cepeda C., Gil-Sotres F., Leiros M. C. 2007. Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. *Soil Biology and Biochemistry* 39, 311–319 10.1016/j.soilbio.2006.08.002.

Vandana, J.L. 2012. Urease and phosphomonoesterase activities in soil – their distribution, kinetics and influence of management practices on their activities. *Ph.D. Thesis.* Acharya N G Ranga Agricultural University, Hyderabad

Wallenstein MD, Mcmahon SK, Schimel JP 2009 Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Global Change Biology*, 15, 1631–1639.

\*\*\*\*\*