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Effect of Temperature on Alkaline Phosphatase Activity in Alfisols and Vertisols of Andhra Pradesh

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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. The temperature increase caused by global warming 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 20°C to 90°C. 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 (Q_{10}) were calculated in the temperature range of 20 to 90°C. 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 isincreased due to various changes caused by global warming and other aspects, it has a profound influenceon 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). 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 soil phosphatases solubilise organic phosphates and make them available for plant growth and the soil becomes rich in soluble phosphate which behaves like an index of soil fertility which mostly depends on optimum temperature (Porter and Gawith, 1999). 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 1000 ml with distilled water. ModifiedUniversal Buffer (pH 6.5): 200 ml of MUB stock was transferred to 1000 ml beaker and kepton a magnetic stirrer and the pH of the solution was adjusted to 6.5 with 0.1N HCland volume was made up to 1000 ml with distilled water.

Modified Universal Buffer (pH 11): 200 ml of MUB stock was transferred to 1000 ml 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 withdistilled 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 in40ml of MUB pH 11 (for assay of alkaline phosphatase) and the solution was diluted to50 ml with MUB of the same pH. The solutionwas wrapped with carbon paper and stored ina refrigerator.

Calcium chloride (0.5M): This was prepared by dissolving 73.5g of $CaCl_2.2H_2O$ in distilledwater 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: Primarystock 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 wereprepared. 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, 4ml 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 ± 0.5°C in BOD incubator. To these, 1 ml of 0.5M $CaCl_2$ 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 thesoil 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 $CaCl_2$ and 4 ml of 0.5M NaOH. Corrections were made forcontrol / 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 – 60°C and thereafter activity decreased slowly till 70°C and rapidly decreased with further increase in temperature to 90°C. Denaturation occurred beyond 60°C 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 20-60°C and thereafter the activity decreased to 78.14 µg of 4-nitrophenol g^{-1} soil h^{-1} at 70°C and further decreased to 21.48 µg of 4-

nitrophenol g^{-1} soil h^{-1} at 90°C. 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 20-60°C and there after the activity decreased to 367.74 µg of 4-nitrophenol g^{-1} soil h^{-1} at 70°C and further increased to 80.98 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90°C. 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 60°C was decreased due to loss of thermal stability of enzyme.

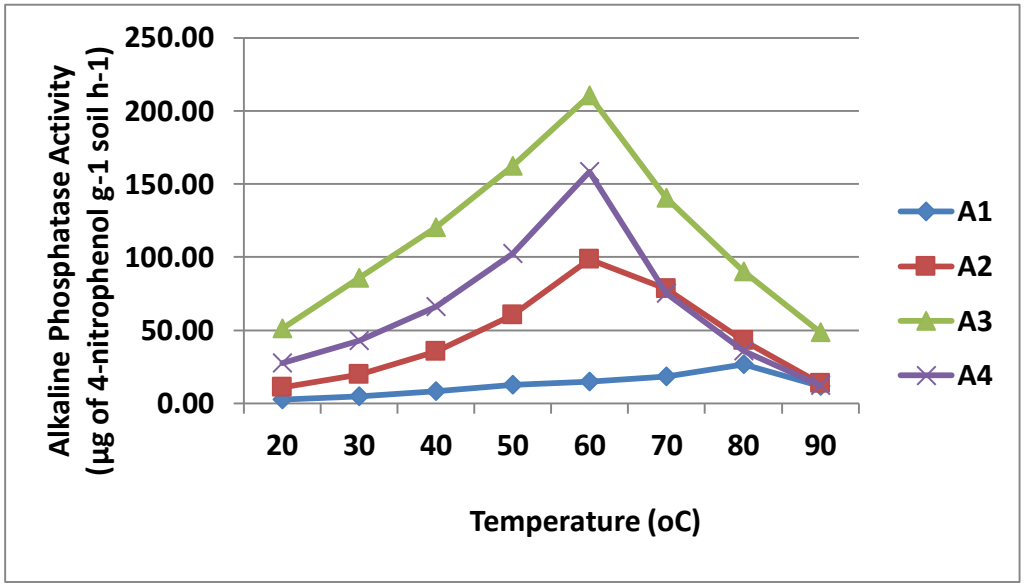

Fig.1 Effect of temperature on soil acid phosphatase activity in alfisols

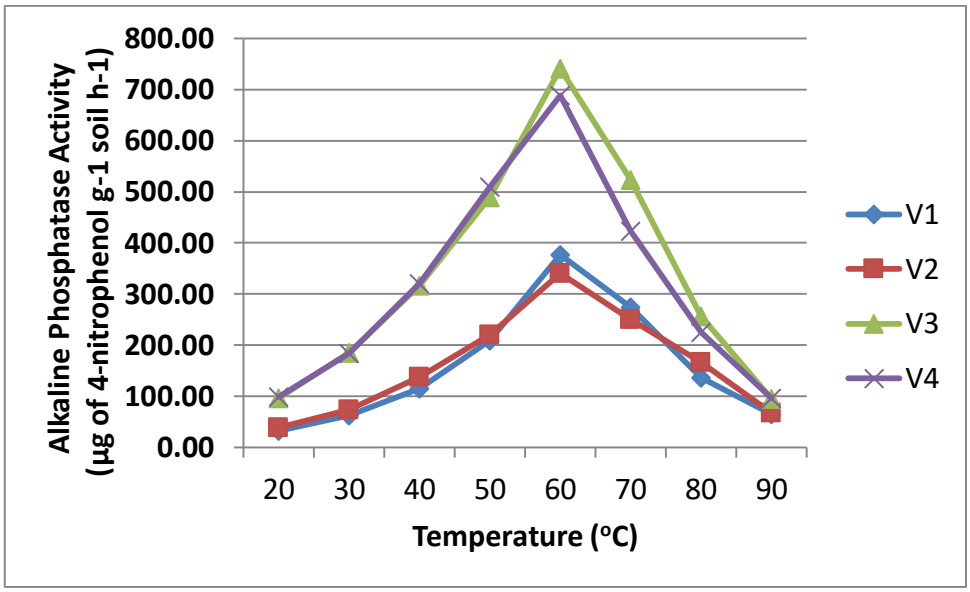

Fig.2 Effect of temperature on soil acid phosphatase activity in vertisols

The temperature coefficient values (Q_{10}) werecalculated in the temperature range of 20 to 90°C 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 (Q_{10}) of Alkaline phosphatase in alfisols

Temperature range (°C)	Temperature Coefficient Values (Q_{10}) 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 (Q_{10}) of Acid phosphatase in Vertisols

Temperature range (°C)	Temperature Coefficient Values (Q_{10}) 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 on soil enzyme activities, changing enzyme kinetics , stability, substrateaffinity and enzyme production because it can influence the size and activity of microbial biomass. Alkaline phosphatase activity of soils increased with temperature from 20°C to 60°C and decreased constantly with further increase in temperature to 90°C. Similarly, the temperaturedependence of soil hydrolase activities was described by Arrhenius equation (Cepeda *etal.,* 2007). They measured the Q_{10} of nine different enzymes in three different soils and found that the Q_{10} at 20°C 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 (1994) 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 10^0C rise in temperature the rate of the reactionapproximately increase by two folds. The rate of enzyme catalyzed reaction increases as the temperature increases until optimumtemperature 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 soilparticulate 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 reactionsmay 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 ^{o}C 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, 1994 and Srinivas and Raman, 2000). Recent increases in climate variability might have affected crop yields in countries across Europe since aroundthe mid-1980s (Porter & Semenov 2005) causing higher inter-annual variability in wheat yields which suggested a high risk of wheat crop in Spain. This suggested changes in temperature not only effectthe 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.

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References

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. (https://doi.org/10.1016/0038-0717(88)90003-X).

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. (https://doi.org/10.2136/sssaspecpub35.c7).

Dora, A.S., Domuta, C., Ciobanu, C. and Sandor, M. 2008. Field management effects on soil enzyme activities. *Romanian Agricultural Research*. 25: 61-68. (https://www.incda-fundulea.ro/rar/nr25/rar25.11.pdf)

Eivazi, F. and Tabatabai, M. A. 1977. Phosphatases in soils. *Soil Biology and Biochemistry*. 9: 167-172. (DOI:10.1016/0038-0717(77)90070-0)

Fenner, N., Freeman, C. and 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 (https://doi.org/10.1016/j.soilbio.2005.02.032).

Koch, O., Tscherko, D. and Kandeler, E. 2007. Temperature sensitivity of microbialrespiration, nitrogen mineralization, and potential soil EAs in organic alpine soils. *Global Biogeochemistry Cycles* 21, 1–11. (DOI:10.1029/2007GB002983).

Porter, J. R. and Semenov, M. A. 2005. Crop responses to climatic variation. Phil.Trans. R. Soc. B 360, 2021–2035 (**https://doi.org/10.1098/rstb.2005.1752**).

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. (**CABI Record Number**: **20023022765**)

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 (https://doi.org/10.1016/0038-0717(69)90012-1).

Tabatabai, M.A. 1994. Microbiological andbiochemical 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. (https://doi.org/10.2136/sssabookser5.2.c37).

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. (https://doi.org/10.1016/j.soilbio.2006.08.002).

Wallenstein, MD., Mcmahon, SK. and Schimel, J.P. 2009. Seasonal variation in enzyme activities and temperature sensitivitiesin Arctic tundra soils. *Global Change Biology*. 15; 1631–1639. (DOI:10.1111/j.1365-2486.2008.01819.x).

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