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Influence of Yemperature and Q_{10} Values on Alkaline Phosphatase Activity in Vertisols of Andhra Pradesh

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ABSTRACT

The enzyme phosphatase plays an important role in the mineralization of organically bound P that leads to absorption of P by the plants. Alkaline phosphatase belong to the group of phosphomonoesterases play an important role in catalyzing several important reactions necessary for the life processes of microorganisms in soils and thereby stabilizing the soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling. When the temperatures are increased due to various changes caused by global warming that 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 increase in temperature the enzymes get denatured and results in a decreased nutrient availability and indirectly the productivity. To study the effect of temperature on soil alkaline phosphatase activity, ten Vertisol sampleswere collected and laboratory incubation studies were carried out at different temperatures ranging from 20°C to 90°C. The average alkaline phosphatase activity (µg of 4-nitrophenol g^{-1} soil h^{-1}) varied from 69.4 to 542.2 with the increased temperature from 20-60°C and there after the activity decreased at 90 °C. Among the samples, S5 recorded higher activity of 780.4 µg of 4-nitrophenol g^{-1} soil h^{-1} followed by S8 (602.6), S7(594.5) and S1 (575.4). The temperature coefficient values (Q_{10}) were calculated in the temperature range from 20 to 90°C for alfisols and the average value varied from 0.50 to 1.74.

Key words: *Alkaline phosphatase, Incubation study,Temperature and Vertislols*

Agriculture is influenced by climate change, temperature being one of the key components. Soil enzymes play a key role in the overall process of organic matter decomposition and organic nitrogen in soil system 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 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 facts of soil enzymes the *in situ* behaviour of soil enzymes in heterogeneous environment of the soil system in respect of their thermal sensitivities, pH effects, kinetics and moisture effects are of prime importance. Hence the present investigation was undertaken to study the effect of temperature on soil alkaline phosphatase activity in Vertisols of Andhra Pradesh.

MATERIAL AND METHODS

The procedure of Eivazi and Tabatabai (1977) was adopted for the assay of alkaline phosphatases activity in soils. Soil samples belonging to ten Vertisols 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 11.0): 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 11.0 with 0.1N NaOH 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 disodium salt of p-nitrophenyl phosphate in 40ml of MUB pH 6.5 (for assay of acid 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 $CaCl_2.2H_2O$ 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 11.0 (for assay of alkaline 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 the soil suspension was filtered through Whatman No. 42 filter paper. The absorbance of yellow color of 4-nitrophenol liberated due to hydrolysis of the substrate by alkaline phosphatase was measured at 420 nm. Controls were run by the 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 for control / blank values?

RESULTS AND DISCUSSION

The results on the effect of temperature on soil acid phosphatase activity in vertisols are presented in table 1 and depicted graphically in Figure: 1. Alkaline phosphatase activity of all soils used in study increased with increase in temperature from 20 – 60°C and thereafter activity decreased slowly upto 70°C and rapidly decreased with further increase in temperature from 80 to 90°C. Denaturation occurred beyond 60°C in vertisols under study.

The average alkaline phosphatase activity varied from 69.4 to 542.2 µg of 4-nitrophenol g^{-1} soil h^{-1} with the increased temperature from 20-60°C and there after the activity decreased to 306.5 µg of 4-nitrophenol g^{-1} soil h^{-1} at 70 °C, 153.1 at 80°C and finally decreased to 77.8 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 °C. Among the vertisols, S5 recorded higher activity of 780.4 µg of 4-nitrophenol g^{-1} soil h^{-1} followed by S8 (602.6), S7 (594.5) and S1 (574.1). The alkaline phosphatase activity beyond optimum temperature of 60°C was decreased due to loss of thermal stability of enzyme.

Temperature Quotient values (Q_{10})

The temperature coefficient values (Q_{10}) were calculated in the temperature range of 20 to 90°C for the Vertisols are presented in table 2. The average value varied from 0.50 to 1.74 over the range from 20-90°C. Among the soil samples, Q_{10} values varied from 1.03 to 1.31. The highest value was recorded in S2 followed by S3, S8 and S1.

Temperature has a profound effect and it 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. Acid phosphatase activity of soils increased with temperature from 20°C to 70°C and decreased constantly with further increase in temperature to 90°C (Rao, 1989 and Vandana, 2012). The temperature dependence of soil hydrolase

activities was described by Arrhenius equation (Cepeda *et al.,* 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.

The activity of any chemical reaction increases with temperature, for every 10^0C rise in temperature the rate of the reaction approximately increased 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 might 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; Cepeda *et al.,* 1988(check the year in references) and Wallenstein *et al.,* 2009).

It is known that the temperature needed to deactivate enzymes in soils is about 10 °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, 1982;Srinivas *et.al.* 2000 and Vandana , 2012). Changes in temperature not only effect the enzyme production but also effect enzyme degradation rates in the environments. Biological responses include changes in enzyme production rates with shifts in microbial population and composition. The variation in these values may be due to heterogeneity in composition and the state of enzymes at temperature above 40°C. Recent increase in climate variability might have affected crop yields in countries across in Europe (Porter & Semenov 2005) causing higher inter-annual variability in wheat yields. This study suggested that such changes in annual yield variability would cause a high-risk in wheat in Spain. Temperature has a profound impact on soil enzyme acid phosphatase activity and its influence on the biogeochemical cycles in the soil.

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Table 1: Effect of temperature on soil alkaline phosphatase activity in Vertisols

Substrate Concentration (mM)	Alkaline phosphatase activity (µg of 4-nitrophenol released g^{-1} soil h^{-1})										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**Mean**
20	60.3	32.4	39.8	58.9	138.0	44.7	65.0	151.4	38.9	64.6	**69.4**
30	104.8	61.7	72.1	102.3	208.9	81.3	120.2	213.8	74.1	104.7	**114.4**
40	181.8	114.8	138.0	177.8	316.2	144.5	204.2	302.0	138.0	169.8	**188.7**
50	323.6	218.8	257.0	309.0	489.8	263.0	346.7	426.6	263.0	275.4	**317.3**
60	575.4	416.9	478.6	537.0	780.4	478.6	594.5	602.6	501.2	457.1	**542.2**
70	302.5	270.4	276.5	324.2	402.6	265.8	325.0	336.0	295.1	266.5	**306.5**
80	141.1	136.2	156.3	155.8	196.9	126.0	167.6	176.4	131.3	143.1	**153.1**
90	74.5	64.7	79.3	72.5	95.1	73.5	88.2	86.2	67.6	76.4	**77.8**
	220.5	**164.5**	**187.2**	**217.2**	**328.5**	**184.7**	**238.9**	**286.9**	**188.6**	**194.7**	

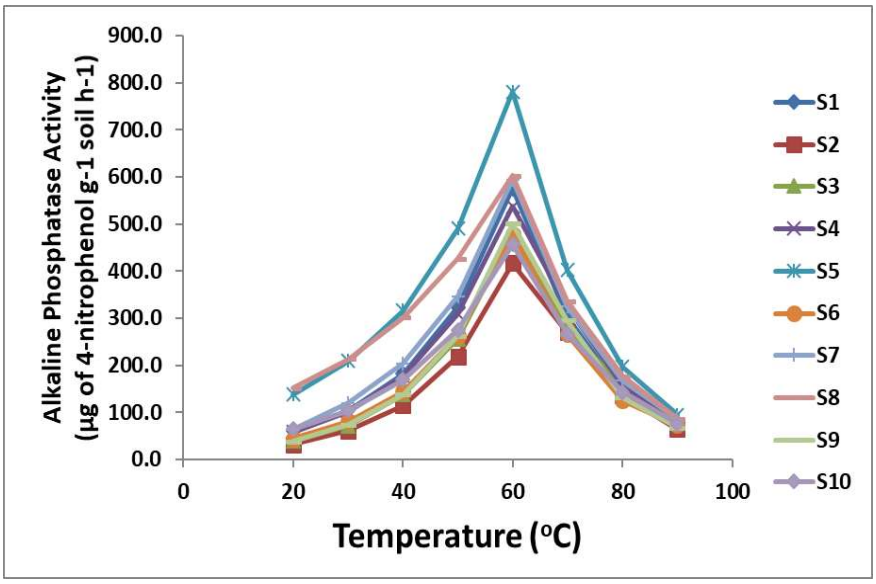

Fig.1 Effect of temperature on soil alkaline phosphatase activity in Vertisols

Table 2 : Temperature coefficient values (Q_{10}) of alkaline phosphatase in Vertisols

Temperature (°C)	Temperature Coefficient Values (Q_{10})										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**Mean**
20-30	1.74	1.90	1.81	1.74	1.51	1.82	1.85	1.41	1.90	1.62	**1.73**
30-40	1.73	1.86	1.91	1.74	1.51	1.78	1.70	1.41	1.86	1.62	**1.71**
40-50	1.78	1.91	1.86	1.74	1.55	1.82	1.70	1.41	1.91	1.62	**1.73**
50-60	1.78	1.91	1.86	1.74	1.59	1.82	1.71	1.41	1.91	1.66	**1.74**
60-70	0.53	0.65	0.58	0.60	0.52	0.56	0.55	0.56	0.59	0.58	**0.57**
70-80	0.47	0.50	0.57	0.48	0.49	0.47	0.52	0.53	0.45	0.54	**0.50**
80-90	0.53	0.48	0.51	0.47	0.48	0.58	0.53	0.49	0.51	0.53	**0.51**
Mean	**1.22**	**1.31**	**1.30**	**1.21**	**1.09**	**1.26**	**1.22**	**1.03**	**1.30**	**1.17**	

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