Int.J.Curr.Microbiol.App.Sci (2018) *7*(9): 2830-2845

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 09 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.709.352

Effect of Temperature on Soil Enzyme Acid Phosphatase

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Keywords

Alfisol, Acid phosphatase, Temperature, Vertisol, Climate change and Productivity

Article Info

Accepted:
20 August 2018
Available Online:
10 September 2018

A B S T R A C T

Soil enzymes play a major role in mineralization of nitrogen, phosphorus and sulphur. mineralization is the process of transformation of organically bound elements into mineral form which will readily taken up by plants and is crucial to plant nutrition and indirectly plays a role agriculture productivity. The enzyme phosphatase plays an important role in providing the plant its nutrition. In most soils, the organically bound P- fraction is higher than the inorganic. Phosphorus uptake by plants requires mineralization of the organic P component by phosphatases to available form. Phosphatases are inducible enzymes that are produced predominantly under conditions of low phosphorus availability. Phosphatases are excreted by plant roots and by microorganisms. Microbial phosphatases dominate in soils. The activity of phosphatase in soil is influenced by the temperature. The abiontic enzymes present in the soil 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. When the temperatures are increased due various changes caused by global warming and other aspects they have a profound influence on soil enzymes and indirectly on agricultural productivity. Every enzyme has its own optimum temperature below the optimum temperature the enzyme activity is less due to inactivation and above the optimum temperature the enzyme activity decreases due to denaturation. Due to increase in temperature the enzymes are denatured and nutrients availability is decreasing and indirectly effecting productivity. To study the effect of temperature on soil enzyme activity four different soils samples were collected and incubation studies were carried out at different temperatures ranging from 20 ^{o}C to 90^{o}C with two Alfisols and two Vertisols. The enzyme activity ranged from 9.2 to 297.5 (µg of 4-nitrophenol g^{-1} soil h^{-1})

Introduction

Agriculture is influenced by climate change, temperature being one of the key components. While farmers are often flexible in dealing 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

is replenished with the mineralization of organic nitrogen. These enzymes play key roles in 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 facets 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 designed for studying the effect of temperature on soil enzyme acid phosphatase activity.

Materials and Methods

The procedure of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) were adopted for the assay of acid phosphatases activity in soils. Two Alfisols and 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 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 disodium salt of p-nitrophenyl phosphate in 40ml of MUB pH 6.5 (for assay of acid phosphatase) and 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 $CaCl_2.2H_2O$ 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 6.5 (for assay of acid phosphatase) and pH 11 (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^{o}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 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 for control / blank values

Results and Discussion

The results regarding to the effect of temperature on soil Acid phosphatases activities are depicted graphically in Figure 1. Acid phosphatases activity of all soils used in the study increased with increase in temperature from 20 – 70^{o}C and then activity decreased slowly till 90^{o}C and rapidly decreased with further increase in temperature to 90^{o}C. Denaturation occurred beyond 70 ^{o}C. for the present study both Alfisols and Vertisols were taken higher activity was observed in Alfisols, the range observed in different soils is as follows in Vertisol I was 11.3 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 20 ^{o}C and increased to 24.1(µg of 4-nitrophenol g^{-1} soil h^{-1} at 30 ^{o}C and further increased to 52.1 µg of 4-nitrophenol g^{-1} soil h^{-1} at 40 ^{o}C and further increased to 101.8 µg of 4-nitrophenol g^{-1} soil h^{-1} at 50 ^{o}C and further increased to 189.3 µg of 4-nitrophenol g^{-1} soil h^{-1} at 60 ^{o}C and further increased to 297.5 µg of 4-nitrophenol g^{-1} soil h^{-1} 70 ^{o}C and then when the temperature is increased beyond their optimum temperature its activity decreased to 152.4 µg of 4-nitrophenol g^{-1} soil h^{-1} at 80 ^{o}C and further decreased to 63 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 ^{o}C and in Vertisol II, the range of enzyme activity was as follows was 9.2 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 20 ^{o}C and increased to 22.8 µg of 4-nitrophenol g^{-1} soil h^{-1} at 30 ^{o}C and further increased to 51.7 µg of 4-nitrophenol g^{-1} soil h^{-1} at 40 ^{o}C and further increased to 98.8 µg of 4-nitrophenol g^{-1} soil h^{-1} at 50 ^{o}C and further increased to 176.3µg of 4-nitrophenol g^{-1} soil h^{-1} at 60 ^{o}C and further increased to 258.2 µg of 4-nitrophenol g^{-1} soil h^{-1} 70 ^{o}C and then when the temperature is increased beyond their optimum temperature its activity decreased to 137.7 µg of 4-nitrophenol g^{-1} soil h^{-1} at 80 ^{o}C and further decreased to 54.2 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 ^{o}C. In case of Alfisol I it was observed that the enzyme activity increased as follows was 12.5 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 20 ^{o}C and increased to 26.9(µg of 4-nitrophenol g^{-1} soil h^{-1} at 30 ^{o}C and further increased to 60.4 µg of 4-nitrophenol g^{-1} soil h^{-1} at 40 ^{o}C and further increased to 133.5 µg of 4-nitrophenol g^{-1} soil h^{-1} at 50 ^{o}C and further increased to 227.6 µg of 4-nitrophenol g^{-1} soil h^{-1} at 60 ^{o}C and further increased to 445.7 µg of 4-nitrophenol g^{-1} soil h^{-1} 70 ^{o}C and then when the temperature is increased beyond their optimum temperature its activity decreased to 202.5 µg of 4-nitrophenol g^{-1} soil h^{-1} at 80 ^{o}C and further decreased to 90.4 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 ^{o}C and in Alfisol II, the range of enzyme activity was as follows was 14.2 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 20 ^{o}C and increased to 29.7 µg of 4-nitrophenol g^{-1} soil h^{-1} at 30 ^{o}C and further increased to 77.8 µg of 4-nitrophenol g^{-1} soil h^{-1} at 40 ^{o}C

and further increased to 152.2 µg of 4-nitrophenol g^{-1} soil h^{-1} at 50 ^{o}C and further increased to 269.2 µg of 4-nitrophenol g^{-1} soil h^{-1} at 60 ^{o}C and further increased to 503.5 µg of 4-nitrophenol g^{-1} soil h^{-1} 70 ^{o}C and then when the temperature is increased beyond their optimum temperature its activity decreased to 260.1 µg of 4-nitrophenol g^{-1} soil h^{-1} at 80 ^{o}C and further decreased to µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 ^{o}C, negligible increased was observed in case of activity because the thermal stability of the enzyme was completely lost. the temperature coefficient of the enzyme was calculated.

The results pertaining to temperature coefficient were given in the Table 1. Temperature coefficient values (Q_{10}) were calculated in the temperature range of 20 to 90^{o}C. These values depend on the type of soil which varied from 0.4 to 2.2 in case of Vertisol I and 0.4 to 2.5 in case of Vertisol II in Alfisol a slight higher temperature Coefficient was observed *i.e.*, 0.4 to 2.2 in Alfisol I and 0.6 to 2.6 in case of Alfisol II.

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. Acid phosphatase activity of soils increased with temperature from 20^{o}C to 70^{o}C and decreased constantly with further increase in temperature to 90^{o}C (Rao, 1989; Srinivas, 1993 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^{o}C exceeded 2.0 only for B-glucosidase in one of the soils. All other soil enzymes in that study had a Q_{10} closer to 1.5, corresponding to an Ea of 0.3 eV.

Fig.1 Effect of temperature on soil acid phosphatase activity

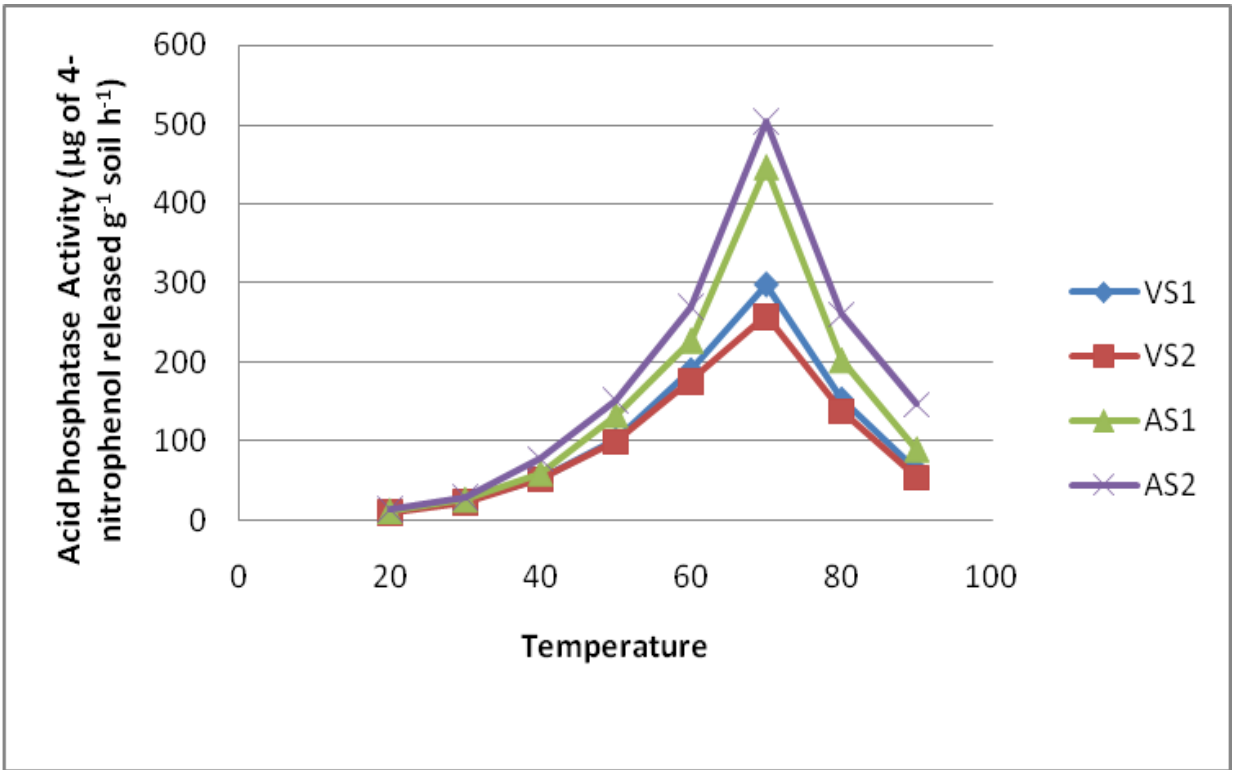

Table.1 Temperature coefficient values (Q_{10}) acid phosphatase

Temperature range (^{o}C)	Temperature Coefficient values (Q_{10}) acid phosphatase			
	VS1	VS2	AS1	AS2
20-30	2.1	2.5	2.2	2.1
30-40	2.2	2.3	2.2	2.6
40-50	2.0	1.9	2.2	2.0
50-60	1.9	1.8	1.7	1.8
60-70	1.6	1.5	2.0	1.9
70-80	0.5	0.5	0.5	0.5
80-90	0.4	0.4	0.4	0.6

The activity of any chemical reaction increases with temperature, for every 10^{0}C 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 know 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, 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. 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^{o}C. When the Q10 values were less than 1 which indicates the deactivation of the enzymes set in at that temperature. Recent increases in climate variability may have affected crop yields in countries across Europe since around the mid-1980s (Porter & Semenov 2005) causing higher inter-annual variability in wheat yields. This study suggested that such changes in annual yield variability would make wheat a high-risk crop in Spain. Even mid-latitude crops could suffer at very high temperatures in the absence of adaptation. In 1972, extremely high summer averaged temperature in the former Soviet Union (USSR) contributed to widespread disruptions in world cereal markets and food security (Battisti and Naylor, 2009).

Temperature has a profound impact on soil enzyme acid phosphatase activity and it influence the biogeochemical cycles in the soil.

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How to cite this article:

Aruna Kumari, J., P.C. Rao, G. Padmaja and Madhavi, M. 2018. Effect of Temperature on Soil Enzyme Acid Phosphatase. *Int.J.Curr.Microbiol.App.Sci.* 7(09): 2830-2845.
doi: https://doi.org/10.20546/ijcmas.2018.709.352