Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6 Special issue [3] 2017: 282-286

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804
NAAS Rating 4.95

FULL LENGTH ARTICLE **OPEN ACCESS**

Effect of temperature on soil enzyme alkaline phosphatase

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ABSTRACT
Enzyme activities may have the potential to be used as indicators of soil quality, sustainability and changes in biogeochemical function due to management or perturbations. In general, soil enzyme activities can be used to characterize abundance and metabolic activity of soil microbes, whereas kinetic parameters are used to describe catalytic activity, origin, and substrate affinity of the enzymes. Soil enzyme activity provides an indication of its amount and its overall contribution in soil, while enzyme kinetics study can provide useful information regarding their origin, existing status and catalytic properties, state and behaviour of soil. Kinetics of enzymes is influenced by temperature. 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 acitivity of phosphatase in soil is influenced by the temperature. 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 and 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. 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 affecting productivity. To study the effect of temperature on soil enzyme activity, four different soil 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 18.7 to 520.3 (µg of 4-nitrophenol g^{-1} soil h^{-1}) in Vertisols and 27.4 to 601.4 (µg of 4-nitrophenol g^{-1} soil h^{-1}) in Alfisols.
***Key words**: Alfisol, Alkaline phosphatase, Temperature, Vertisol, Climate change and Productivity*

Received 29.07.2017 Revised 09.08.2017 Accepted 23.08. 2017

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 and 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, 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. In the soils of arid and semi arid tropics, organisms can assimilate only dissolved phosphate and therefore, phosphatase 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 and sometime unavailable forms of organic P into assimilable

phosphate. Thus, phosphatase production depends on a combination of P demand from plants and microbes, available organic P substrate and P limitation of the soil.

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 aluminium 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 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). Phosphorus is present in soil in several organic and inorganic forms, and only a small fraction of P_{org} is susceptible to release available phosphate after phosphatase reaction (**Johnson** *et al* 2003).

MATERIAL AND METHODS

The procedure of **Tabatabai and Bremner** (1969) and **Eivazi and Tabatabai** (1977) was 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 malice 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 the volume was made upo 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 upto 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): It was prepared by dissolving 73.5g of $CaCl_2.2H_2O$ in distilled water and made upto 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 upto 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°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

The results regarding the effect of temperature on soil Alkaline phosphatases activities are depicted graphically in Figure (1). Alkaline phosphatases activity of all soils used in the study increased with increase in temperature from 20 – 70°C and then activity decreased slowly till 90°C and rapidly decreased

with further increase in temperature to 90°C. Denaturation occurred beyond 70 °C for the present study in both the Alfisols and Vertisols. Higher activity was observed in Alfisols, the range observed in different soils is as follows: In Vertisol I was 21.9 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 20 °C and increased to 43.6(µg of 4-nitrophenol g^{-1} soil h^{-1} at 30 °C and further increased to 85.51 µg of 4-nitrophenol g^{-1} soil h^{-1} at 40 °C and further increased to 169.4 µg of 4-nitrophenol g^{-1} soil h^{-1} at 50 °C and further increased to 314.3 µg of 4-nitrophenol g^{-1} soil h^{-1} at 60 °C and further increased to 520.3 µg of 4-nitrophenol g^{-1} soil h^{-1} at 70 °C and then when the temperature is increased beyond their optimum temperature, its activity decreased to 130.5 µg of 4-nitrophenol g^{-1} soil h^{-1} at 80 °C and further decreased to 78.4 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 °C .

In Vertisol II, the range of enzyme activity was 18.7 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 20 °C and increased to 36.5 µg of 4-nitrophenol g^{-1} soil h^{-1} at 30 °C and further increased to 71.4 µg of 4-nitrophenol g^{-1} soil h^{-1} at 40 °C and further increased to 147.8 µg of 4-nitrophenol g^{-1} soil h^{-1} at 50 °C and further increased to 280.8µg of 4-nitrophenol g^{-1} soil h^{-1} at 60 °C and further increased to 498.1 µg of 4-nitrophenol g^{-1} soil h^{-1} at 70 °C and then when the temperature increased beyond their optimum temperature, its activity decreased to 99.2 µg of 4-nitrophenol g^{-1} soil h^{-1} at 80 °C and further decreased to 67.3 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 °C . In case of Alfisol I, it was observed that the enzyme activity was 27.4 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 20 °C and increased to 54.8 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 30 °C and further increased to 101.8 µg of 4-nitrophenol g^{-1} soil h^{-1} at 40 °C and further increased to 214.1 µg of 4-nitrophenol g^{-1} soil h^{-1} at 50°C and further increased to 348.2 µg of 4-nitrophenol g^{-1} soil h^{-1} at 60 °C and further increased to 560.6 µg of 4-nitrophenol g^{-1} soil h^{-1} at 70 °C and then when the temperature increased beyond their optimum temperature, its activity decreased to 149.3 µg of 4-nitrophenol g^{-1} soil h^{-1} at 80 °C and further decreased to 89.9 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 °C.

In Alfisol II, the range of enzyme activity was 28.2 (µg of 4-nitrophenol g^{-1} soil h^{-1} at 20 °C and increased to 60.9 µg of 4-nitrophenol g^{-1} soil h^{-1} at 30 °C and further increased to 124.6 µg of 4-nitrophenol g^{-1} soil h^{-1} at 40 °C and further increased to 235.7 µg of 4-nitrophenol g^{-1} soil h^{-1} at 50 °C and further increased to 387.6 µg of 4-nitrophenol g^{-1} soil h^{-1} at 60 °C and further increased to 601.4 µg of 4-nitrophenol g^{-1} soil h^{-1} at 70 °C. When the temperature increased beyond their optimum temperature, its activity decreased to 151.9 µg of 4-nitrophenol g^{-1} soil h^{-1} at 80 °C and further decreased to 95.2 µg of 4-nitrophenol g^{-1} soil h^{-1} at 90 °C, negligible increase 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 are given in the Table (1). 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 0.3 to 2.0 in case of Vertisol I and 0.2 to 2.1 in case of Vertisol II. In Alfisol, a slight higher temperature coefficient was observed i.e., 0.3 to 2.0 in Alfisol I and 0.3 to 2.1 in case of Alfisol II. .

DISCUSSION

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°C to 70°C and decreased constantly with further increase in temperature to 90°C (Srinivas, 1993 and Vandana, 2012. As soil hydrolytic enzymes are the main drivers of soil organic matter (SOM), degradation and litter decomposition, the dependence of these enzymes on global changes including warming, precipitation, drought and associated soil moisture will assist in understanding the relationships among SOM stock, global carbon cycle and microbial nutrient demand. The rate of extracellular enzyme production is more responsive to temperature than the kinetics of the enzymes themselves. It is not currently possible to directly measure enzyme production rates in soils (Wallenstein and Weintraub 2008). The effect of temperature on enzyme activity is not a simple dependence. Biochemical activity, as well as microbial activity, is modified by the changing temperature of the soil (Jefferies *et al*., 2010; Papatheodorou *et al*., 2004; Vorboyova *et al*., 1996).

Soil phosphatases solubilise inorganic and 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 (Cookson, 2002; Roldan *et al*., 2005; Garcia-Ruiz *et al*., 2008; Alvarez *et al.,* 2012., Porter and Gawith,1999.). Biochemical processes are much more intense under the conditions of variable temperature, which happens in the soil environment. A consequence of climate change could be the limited availability of substrates to both intracellular and extracellular enzymes (Borowik *et al*., 2014; Davidson and Janssens, 2006; Kodaira, 2014). Acosta-Marinez and Tabatabai (2002) showed that arylamidase activity increased with a temperature increase from 20 to 60°C. Higher temperature caused denaturation of this enzyme. According to D'Amico *et al*. (2006) temperature plays a major role in the

release of nutrients and also plays a major role in productivity as it influence physiological aspects of plants.

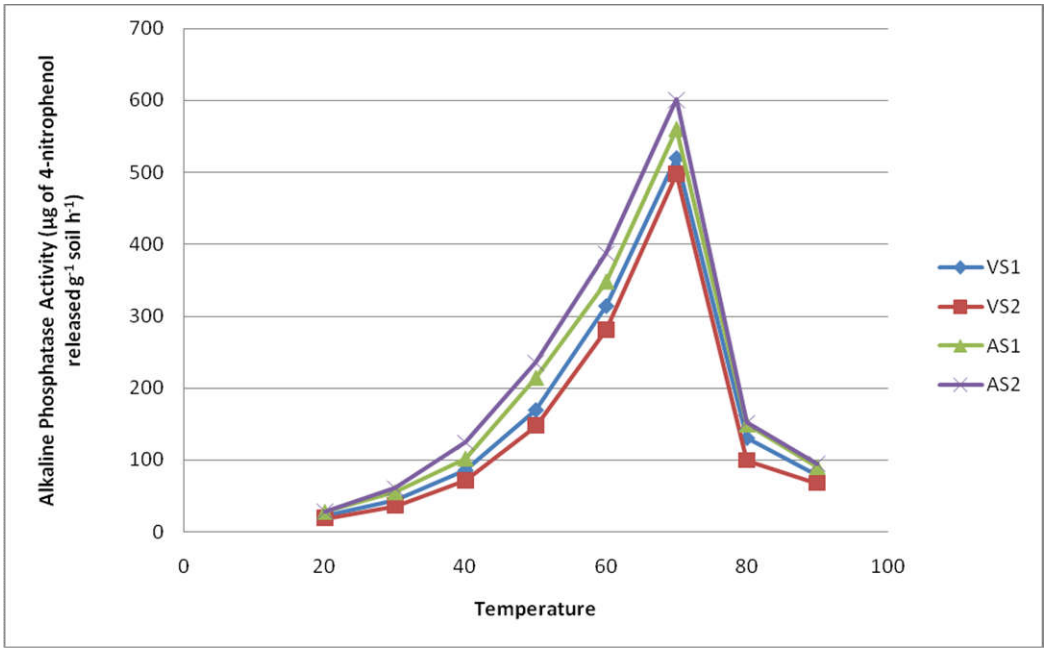

Figure 1. Effect of Temperature on Soil Alkaline Phosphatase Activity

Table 1. Temperature Coefficient values (Q_{10}) alkaline phosphatase

Temperature range (°C)	Temperature Coefficient values (Q_{10}) alkaline phosphatase			
	VS1	VS2	AS1	AS2
20-30	2.0	2.0	2.0	2.2
30-40	2.0	2.0	1.9	2.0
40-50	2.0	2.1	2.1	1.9
50-60	1.9	1.9	1.6	1.6
60-70	1.7	1.8	1.6	1.6
70-80	0.3	0.2	0.3	0.3
80-90	0.6	0.7	0.6	0.6

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CITATION OF THIS ARTICLE

J. Aruna Kumari, P.C.Rao, A. Padmasri and B.Anil Kumar. Effect of temperature on soil enzyme alkaline phosphatase .Bull. Env. Pharmacol. Life Sci., Vol 6 Special issue [3] 2017: 282-286