

## Effect of Physico-Chemical Properties on Soil Enzyme Acid Phosphatase Activity of Some Soils in Vegetable Growing Soils of Ranga Reddy District of Telangana State, India

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### ABSTRACT

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Physico- chemical properties of soil have profound influence on soil enzyme acid phosphatases activity. To study the distribution of soil enzyme acid phosphatase and influence of physico- chemical properties on soil enzyme acid phosphatases activity forty soil samples were collected from different villages of Ranga Reddy district of Telangana state. These samples were analysed for the physicochemical properties like pH, EC, available nutrients, texture and organic carbon and soil enzyme activity was assayed. Acid phosphatase activities of the soil expressed as  $\mu\text{g}$  of 4-nitrophenol released  $\text{g}^{-1}$  soil  $\text{h}^{-1}$  ranged from 18.93 to 51.2 with an average value of 31.79. The pH ranged from 5.7 to 8.9, electrical conductivity from 0.1 to 1.23  $\text{dSm}^{-1}$  and organic carbon from 0.13 to 1.48 %. The available Nitrogen varied from 201.5 to 472.5  $\text{kg ha}^{-1}$ . The available  $\text{P}_2\text{O}_5$  status in the soils varied from 11.6 to 79.1  $\text{kg ha}^{-1}$ . The range of available  $\text{K}_2\text{O}$  ranged from 118 to 411  $\text{kg ha}^{-1}$ . Acid phosphatase significantly, positively correlated with organic carbon ( $r = 0.735^{**}$ ) and available P ( $r = 0.776^{**}$ ). Acid phosphatase activity did not show any significant correlation either with silt, clay and pH.

### Introduction

Soil is important among all the components of terrestrial ecosystems and is a main source of production in agriculture. Since soil is a dynamic system there is a continuous reaction between soil minerals, organic matter and organisms. These reactions influences physio-chemical and biological properties of soil. Soil phosphatases is one of enzyme which plays a major role in the mineralisation processes (dephosphorylation) of organic P substrates.

The most extensively studied group among the phosphatases in soils is the phosphomonesterases, acid phosphatase (EC

3.1.3.2). In agricultural soils phosphatase activity is affected by soil properties, crop plants and farming systems.

Enzymes catalyze all biochemical reactions and are an integral part of nutrient cycling in the soil. Soil enzymes are believed to be primarily of microbial origin but also originate from plants and animals (Tabatabai, 1994). They are usually associated with viable proliferating cells, but enzymes can be extracted from both living and dead cells (Tabatabai, 1994). Soil enzymes are considered to be indicative measures of soil fertility (Zahir *et al.*, 2001).

Since enzyme activity is linked with several ecosystem processes including soil formation, organic matter transformation and bioremediation activities, it is important to understand the different physico-chemical factors affecting the enzyme activities.

## Materials and Methods

Forty soil samples were collected from different mandals of Ranga Reddy district of southern Telengana zone. These samples were air dried and passed through 2 mm sieve before use. These soils samples were analyzed for their different soil properties viz. physical, physico-chemical and chemical properties by using standard procedures (Jackson, 1973). Soil pH-The pH of soil was determined in 1:2.5 soil- water ratio as described by Jackson (1973) using a digital combined glass electrode pH meter (model DI-707). Electrical Conductivity ( $\text{dSm}^{-1}$ ) -The EC was determined in 1:2.5 ratio of soil to water extract as detailed by Jackson (1973) using a digital conductivity bridge and expressed in  $\text{dSm}^{-1}$  (model DI – 909). Organic Carbon (%) Organic carbon in soil was estimated by Walkley and Black (1934) method and as described by Jackson (1973). It is expressed as percentage of organic carbon. Mechanical Analysis-Mechanical composition of soils was determined by Bouyoucos hydrometer method (Bouyoucos, 1962). The relative proportion of sand, silt and clay of soils were determined to describe their textural classes were carried out with the help of international triangle (Singh, 1980). Available Nitrogen ( $\text{kg ha}^{-1}$ ) -The available nitrogen was determined by Macrokjeldhal distillation method using alkaline potassium permanganate method as described by Subbaiah and Asija, (1956) and modified alkaline  $\text{KMnO}_4$  method by Sahrawat and Barford and expressed as  $\text{kg ha}^{-1}$ . Available Phosphorus ( $\text{kg ha}^{-1}$ ) -The available phosphorus was determined by Olsen's method (1954). The blue colour was

developed by using L-ascorbic acid in this method. The intensity of blue colour developed was measured by using spectrophotometer at 420 nm and expressed as  $\text{kg ha}^{-1}$ . Available Potassium ( $\text{kg ha}^{-1}$ ) -The available Potassium in soil was estimated by using neutral normal ammonium acetate extractant (Jackson, 1967) by using Elico flame photometer and expressed as  $\text{kg ha}^{-1}$ .

The procedure of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) were adopted for the assay of acid and alkaline phosphatases respectively. 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. 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  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  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  $1000 \mu\text{g ml}^{-1}$  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

$\mu\text{g ml}^{-1}$  and  $20 \mu\text{g ml}^{-1}$  solutions were prepared. Working standards of 1, 2, 3, 4, 5, 6, 7, 8, 9 and  $10 \mu\text{g ml}^{-1}$  were prepared from  $20 \mu\text{g ml}^{-1}$  stock and the absorbance of these standards were recorded at 420nm in spectrophotometer. This was used for the standard curve. To 1 g of soil sample taken in glass tubes, 4 ml of modified universal buffer pH 6.5 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  $37 \pm 0.5^\circ\text{C}$  in BOD incubator. To these, 1 ml of 0.5M  $\text{CaCl}_2$  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  $\text{CaCl}_2$  and 4 ml of 0.5M NaOH. Corrections were made for control / blank values.

Correlation study was carried out to find the relationship between soil properties and soil enzyme activities.

## Results and Discussion

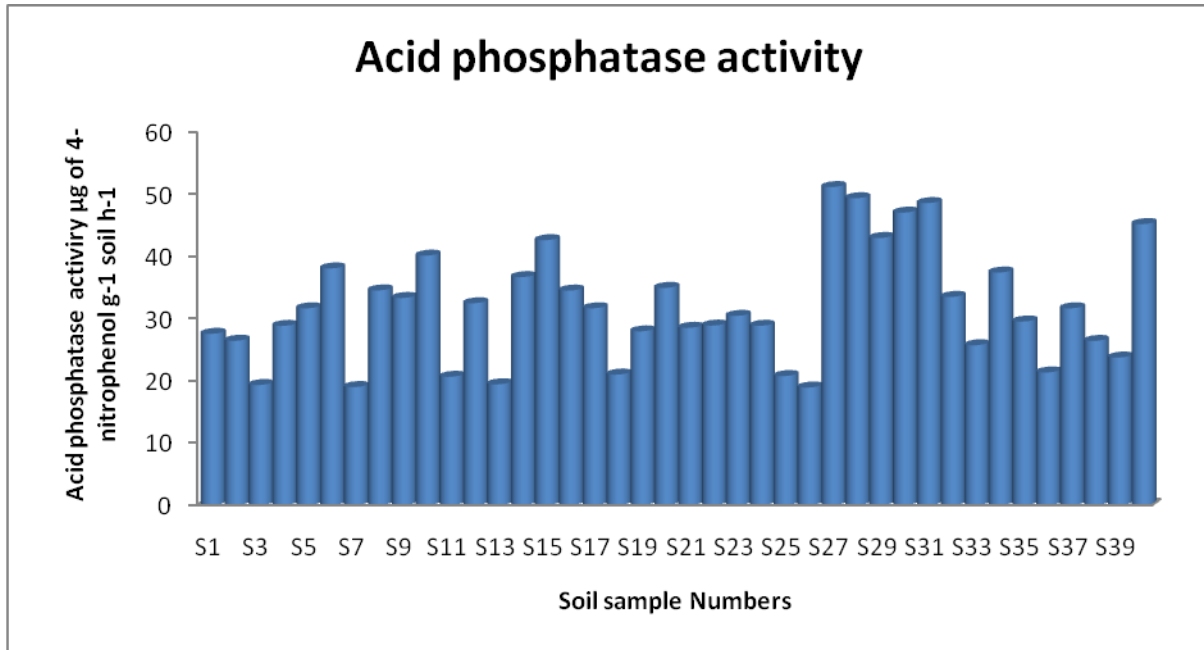
The results of the analysis of these initial soil samples varied in texture from clayey to sandy loam. The pH ranged from 5.7 to 8.9, electrical conductivity from 0.1 to  $1.23 \text{ dSm}^{-1}$  and organic carbon from 0.13 to 1.48 %. The available N varied from 201.5 to  $472.5 \text{ kg ha}^{-1}$ . The available  $\text{P}_2\text{O}_5$  status in the soils varied from 11.6 to  $79.1 \text{ kg ha}^{-1}$ . The range of available  $\text{K}_2\text{O}$  ranged from 118 to  $411 \text{ kg ha}^{-1}$ . These results showed that soil samples collected exhibited a wide variation in their

properties. They are slight acidic to alkaline in reaction and non – saline in nature. Acid phosphatases activity ranged from 18.93  $\mu\text{g}$  of 4-nitrophenol released  $\text{g}^{-1} \text{ soil h}^{-1}$  in sample 26 to 51.2  $\mu\text{g}$  of 4-nitrophenol released  $\text{g}^{-1} \text{ soil h}^{-1}$  in sample 27. Acid phosphatase significantly, positively correlated with organic carbon ( $r = 0.735^{**}$ ) and available P ( $r = 0.776^{**}$ ). Acid Phosphatases activity did not show any significant correlation either with silt, clay and pH. The higher correlation of phosphatase activity with organic carbon content could be due to the fact that the organic matter is the seat of microbial population and activity

These results on soil properties represented here are similar to the results obtained by Kalyani, (2011), Vandana, (2012) and Pavani, (2015). Vandana, (2012) also showed significant positive correlation of phosphatase with organic carbon and insignificant correlation with pH and clay. Sriramachandrasekhar *et al.*, (1997). Gianfreda *et al.*, (2005) found a significant positive correlation of urease and phosphatase with organic carbon content, available nitrogen and available phosphate. The relationship of phosphatase activity to soil organic phosphorous content has been the subject of several studies.

Khaziev and Burangulova (1965) recorded inverse relationships between organic phosphorous contents and soil nuclease, phytase, glyecrophosphatases and phosphatase activities. Nannipieri *et al.*, (1973) observed significant positive correlation between phosphatase activity and organic matter content in soil. While Harrison, (1983) reported a significant positive relationship between phosphatase activity and organic carbon content in woodland soils. Speir, (1977) showed that phosphatase activity significantly correlated with organic carbon (Fig. 1).

**Fig.1** Soil enzyme activity in various samples



**Statistical analysis of various physico chemical properties of soil with soil enzyme acid phosphatase**

**CORRELATION MATRIX**

	sand	silt	clay	Ph	organic carbon	ec	avail nitrogen
sand	1.00000	-0.55848 ***	-0.92708 ***	-0.28989	-0.04715	-0.30091	-0.23688
silt		1.00000	0.20901	-0.01817	0.11858	0.21321	0.20516
clay			1.00000	0.35098 *	-0.01361	0.25789	0.17024
Ph				1.00000	0.02773	-0.06303	0.23124
organic carbon					1.00000	-0.16325	0.55017 ***
ec						1.00000	0.10443
avail nitrogen							1.00000

	p205	k20
sand	0.00902	0.01964
silt	-0.11440	-0.06092
clay	0.02842	0.01001
Ph	0.19901	0.06993
organic carbon	0.40652 **	0.02545
ec	0.08367	-0.20490
avail nitrogen	0.55392 ***	0.07504
p205	1.00000	0.04311
k20		1.00000

Yi Variable = acid p		t Value	Probability
Y3 = 30.9524 +0.01849 x	sand	0.1843	0.8547
Y3 = 33.1372- +0.07208 x	silt	0.2736	0.7859
Y3 = 32.5556- +0.01864 x	clay	0.1577	0.8755
Y3 = 31.7436 +0.05602 x	Ph	0.0289	0.9771
Y3 = 21.1546+19.65897 x	organic carbon	6.6929	0.0000 ***
Y3 = 33.2282- +4.42929 x	ec	0.5135	0.6106
Y3 = 13.3567 +0.06352 x	avail nitrogen	3.6895	0.0007 ***
Y3 = 18.2598 +0.37099 x	p205	7.5903	0.0000 ***
Y3 = 31.1187 +0.00425 x	k20	0.2716	0.7874

However, there had also been reports of non-significant correlation (Sanikidze *et al.*, 1973) and even a negative relationship (Kozlov *et al.*, 1973) between phosphatase activity and organic carbon content of the soil. From the literature, soil phosphatase activity can be related to soil organic matter (Kiss *et al.*, 1974, Nannipieri *et al.*, 1973, Jordan and Kremer 1994, Aon and Colaneri, 2001 and others), total nitrogen (Speir, 1978, Aon and Colaneri, 2001 and others), soil organic phosphorus content (Gavrilova *et al.*, 1973 and others).

Mamytov *et al.*, (1974) found that in certain cultivated soils of Russia, alkaline phosphatase activity was inversely related, whereas, acid and neutral phosphatase activities were directly related to organic phosphorous content. Soil phosphatase activity had been reported to be significantly correlated to total nitrogen also, presumably because it is closely related to organic carbon itself (Harrison, 1983). Chhonkar and Tarafdar, (1984) reported that the phosphatase activity was significantly and positively correlated with organic carbon content, organic phosphorus and bacterial population but it had a negative relationship with a pH of soil.

Soil phosphatase activity has also been shown to vary with soil depth (Harrison, 1983), season (Harrison and Pearce, 1979), and with the type of plants present (Harrison, 1983).

Soil phosphatase correlated with other chemical and biological properties of the soils. Bonmati *et al.*, (1991) assert that phosphatase activity in soils from experimental plots, after storage at room temperature for 1 year. before being analysed, was correlated with protease, total N and organic C. Sahrawat (1983) measured the biological activity in crop rotation using microbial biomass and enzyme activity as parameters. Microbial biomass and alkaline phosphatase in barley fields were correlated to pH values and the organic C content of the soil. The influence of pH on the rate of phosphatase activity has also been described by Herbien and Neal, (1990). Acosta-Martinez and Tabatabai, (2000) described the correlations between phosphatases activity and pH as the effect of eight lime application rates. The wide reports in the 1980s by Chhonkar and Tarafdar, (1984) described positive correlations of phosphatase activity with organic carbon, organic phosphorus and bacterial populations, and a negative relationship with soil pH. Nahas *et al.*, (1994) the activity of acid and alkaline phosphatase correlated with organic matter and total phosphorus content, but not with available phosphorus and organic phosphorus content. The phosphatase producing bacterial population was favoured by the level of available phosphorus.

The results of correlations and linear equations between phosphatase activity and soil characteristics was studied by Sarapatka,

(2003), Positive correlations were found between enzymatic activity and organic carbon, and with nitrogen, and between acid phosphatase activity and total phosphorus. Negative correlations were with the quality of humus (humic: fulvic acids ratio) and available phosphorus, and between acid phosphatase activity and clay content and pH. Zibilske and Bradford, (2003) have found significant correlation between phosphatase activity, extractable P and dissolvable organic carbon. Similar findings were also given by Turner *et al.*, (2002). Their study indicated a link between soluble P in the soil and increased biological and enzyme activity resulting in improvement in soil organic matter content caused by tillage reduction. The results suggest there may be difference in substrate composition that results from conservation tillage and leading to difference in enzyme activity.

The higher correlation of phosphatase activity with organic carbon content could be due to the fact that the organic matter is the seat of microbial population and activity.

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