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Investigations on acid and alkaline phosphatase activity and its relation with soil properties in vertisols of Andhra Pradesh

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Abstract
Physico-chemical properties of soil have a profound influence on soil enzyme acid and alkaline phosphatases activity. The distribution of acid and alkaline phosphatases and the influence of physico-chemical properties on these soil enzymes was assessed in twenty-two soil samples belong to vertisols of the state of Andhra Pradesh. 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 activity of the soil expressed as µg of 4-nitrophenol released g^{-1} soil h^{-1} ranged from 27.3 to 129.3 with an average value of 74.1. Similarly, the alkaline phosphatase varied from 90.2 to 498.7 with a mean value of 206.7 µg of 4-nitrophenol g^{-1} soil h $^{-1}$. The pH of vertisols ranged from 7.6 to 8.7, electrical conductivity from 0.06 to 0.36 dSm^{-1} and organic carbon from 2.9 to 7.8 g kg^{-1}. The available Nitrogen varied from 123 to 267 kg ha^{-1}. The available P status in the soils varied from 14 to 48 kg ha^{-1}. The available K ranged from 159 to 732 kg ha^{-1}. Acid phosphatase was significantly and positively correlated with organic carbon (r = 0.56**) and available P (r = 0.68**). Similarly, Alkaline phosphatase was significantly and positively correlated with organic carbon (r = 0.61**) and available P (r = 0.56**). Both the phosphatases did not show any significant correlation either with silt, clay and pH.

Keywords: Physico-chemical, acid phosphatase, alkaline phosphatase, organic carbon

Introduction
Soil enzymes are important for catalysing innumerable reactions necessary for life processes of microorganisms in soils, decomposition of organic residues, cycling of nutrients and formation of organic matter and soil structure (Dick, 1994) [4]. Although, enzymes are primarily of microbial origin, they can also originate from plants and animals. These enzymes are constantly being synthesized, could be accumulated, inactivated and/or decomposed in the soil, assuming like this, great importance for the agriculture for their role in the recycling of the nutrients (Tabatabai, 1994) [15] The acid phosphatase and alkaline phosphatase are the broad group of phosphomonoesterases play an important role in providing P nutrition to the plant. The organic P in soils exists essentially as esters of phosphoric acid, inositol phosphates, nucleic acids, inositol phosphatases, phospholipids, and other simple esters. Mineralization of these organic compounds to inorganic phosphate is brought about by these enzymes. In agricultural soils, the build-up of these enzymes as well as their activity depends mostly on the soil properties, crop plants and farming systems. Soils of Andhra Pradesh are low to medium in available phosphorus and the hydrolysis of organic P is vital for supply to the plants. The present investigation was undertaken to assess the activity of acid and alkaline phosphatases in vertisols of Andhra Pradesh and to find out their correlation with soil properties**.**

Material and Methods
Twenty-two vertisols with widely varying physic-chemical properties collected from different parts of Andhra Pradesh were used for the study. These soils samples were analyzed in the laboratory for physical, physico-chemical and chemical properties. The pH of soils was determined in1:2.5 soil- water ratio as described by Jackson (1973) [8] using a digital combined glass electrode pH meter (Model DI-707). Electrical Conductivity (dSm^{-1})-The EC was determined in1:2.5 ratio of soil to water extract as detailed by Jackson (1973) [8] using a digital conductivity bridge and expressed in dSm^{-1}. Organic Carbon (mg kg^{-1}) Organic carbon in soil was estimated by Walkley and Black (1934) [18] method and as described by Jackson (1973) [8]. Mechanical composition of soils was determined by Bouyoucos hydrometer method

(Bouyoucos, 1962) [2]. 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) [11]. The available nitrogen (kg ha^{-1}) was determined by Macrokjeldhal distillation method using alkaline potassium permanganate as described by Subbaiah and Asija (1956) [13]. The available phosphorus (kg ha^{-1}) was determined by Olsen's method (1954) [10]. The intensity of blue colour developed by using L-ascorbic acid was measured by using spectrophotometer at 420 nm. The available Potassium (kg ha^{-1}) in soil was estimated by using neutral normal ammonium acetate extractant (Jackson, 1967) [7] by using Elico flame photo meter. The acid phosphatase activity in vertiosls was analysed by the procedure of Tabatabai and Bremner (1969) [14] and alkaline phosphatase by Eivaji and Tabatabai (1977) [5] and details of assay are as follows:

Modified Universal Buffer (MUB) Stock

The stock of MUB was prepared by mixing 12.1 g of Tris (hydroxymethyl) amino methane (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 to1 litre with distilled water.

Modified Universal Buffer (pH 6.5) for assay of acid phosphatase: 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 0.1 NHC land volume was made upto1litre with distilled water.

Modified Universal Buffer (pH 11) for assay of alkaline phosphatase: 200 ml of MUB stock was transferred to 1 litre beaker and kept on a magnetic stirrer and the pH ofthesolutionwasadjustedto11 with 0.1 NNaOH and volume was made up to 1 litre with distilled water. The MUB buffer was wrapped with carbon paper and stored in are frigerator.

P-nitrophenyl phosphate solution (0.025M)

This was prepared by dissolving 0.420 g of disodium salt of p-nitrophenyl phosphate in 40 ml of MUB pH6.5 (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 are frigerator.

Calcium chloride (0.5M)

This was prepared by dissolving 73.5 g of $CaCl_2.2H_2O$ in distilled water and made up to 1 litre.

Sodium hydroxide (0.5M)

20g of sodium hydroxide was dissolved in 700 ml of distilled water and diluted to 1litrewith water.

Standard p-nitrophenol solution

Primary stock solution of 1000 μgml^{-1} of p-nitrophenol was prepared by dissolving 1 g of p-nitro phenol in distilled water and made upto 1 litre. From this, secondary stock of 100 μgml^{-1} and 20 μgml^{-1} solutions were prepared. Working standard s of 1,2,3,4,5,6, 7, 8, 9 and 10 $\mu g\ ml^{-1}$ were prepared from 20 μgml^{-1} stock and the absorbance of these standards were recorded at 420nm in spectrophotometer. This was used for the standard curve.

Procedure

To one gram soil sample taken in glass tubes, 4ml of modified universal buffer pH 6.5 (for assay of acid phosphatase) and 4 ml modified universal buffer pH 11.0 (for assay of alkaline phosphatase) was added separately 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 0.5 M NaOH to deactivate the enzyme and to extract the 4-nitrophenolliberated. The glass tubes were swirled and the soil suspension was filtered through Whatman No. 42 filter paper. The absorbance of yellow color of 4-nitrophenolliberated due to hydrolysis of the substrate by phosphomonoesterase was measured at 420 nm. Controls were run simultaneously following the same procedure except adding 1ml of 4-nitrophenyl phosphate after the addition of 1 ml of 0.5M $CaCl_2$ and 4 ml of 0.5MNaOH. Corrections were made for control/ blank values

Results and Discussion

The physic-chemical properties of vertisols are presented in Table 1. The pH of the soils varied from 7.6 to 8.7 with a mean of 8.3. The EC of soils has a mean of 0.20 dS/m. The organic carbon values varied from 2.9 to 7.8 with a mean value of 5.0 g kg-^{1}. The available N content in soils varied from 123 to 267 with a mean value of 171.5 kg/ha, the available P in soils ranged from 14 to 48 with a mean of 26.5 kg/ha and the available K varied from 159 to 732 with a mean of 411.6 kg/ha. The CEC of the soils varied from 29.6 to 58.7 with a mean of 40.2 cmol (P+)/kg soil. In general, the texture of the soils varied from Clay loam to Clay.

The acid phosphatase and alkaline phosphatase activity in vertiols is presented in Table 1 The acid phosphatase varied from 27.3 to 129.3 with a mean of 74.1 µg of 4-nitrophenol g^{-1} soil h^{-1}. The alkaline phosphatase varied from 90.2 to 498.7 with a mean value of 206.7 µg of 4-nitrophenol g^{-1} soil h^{-1}. The Linear correlation was worked out between the activity of acid phosphatase and alkaline phosphatase with soil properties is presented in Table 2.

The acid phosphatase activity was significantly and positively correlated with organic carbon (0.56**) and available phosphorus (0.68**) in vertiols. Acid phosphatase did not show any significant correlation either with silt, clay and pH. Similarly, the alkaline phosphatase activity was significantly and positively correlated with organic carbon (0.61**) and available phosphorus (0.56**) in vertisols. 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.

Similar results were reported by a number of investigators (Nannipieri *et al*. 1973: Speir, 1977) [9, 12] Chhonkar and Tarafdar (1984) [3] showed a significant and positive relation of phosphatase activity with soil organic carbon and a non-significant correlation with clay content in representative soils of India. Nannipieri *et al.*,(1973) [9] observed significant positive correlation between phosphatase activity and organic matter content in soil. Tarafdar *et al*. (1981) [16] observed a significant and positive correlation of alkaline phosphatase with organic carbon, fungal and bacterial populations with organic carbon in jute growing soils of West Bengal. Harrison (1983) [6] reported a significant positive relationship between phosphatase activity and organic carbon content in woodland soils.

Table 2: Correlation of phosphatase activities with soil properties

Soil properties	Acid phosphatase	Alkaline phosphatase
Organic carbon	0.56**	0.61**
pH	-0.17	0.12
Clay	0.08	-0.08
CEC	0.09	-0.05
Available P	0.68**	0.56**
Available N	0.24	0.33
Available K	0.05	0.04

A positive correlation with acid phosphatase activity and inorganic P was observed by Baligar *et al*. (1988) [1]. Chhonkar and Tarafdar, (1984) [3] 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 and a non-significant correlation with clay in representative soils of India. The results of correlations and linear equations between phosphatase activity and soil characteristics was also studied by Sarapatka, (2003) [20]. Positive correlations were found between enzymatic activity and organic carbon, and with nitrogen, and between acid phosphatase activity and total phosphorus. Zibilske and Bradford, (2003) [19] have found significant correlation between phosphatase activity, extractable P and dissolvable organic carbon. Similar findings were also given by Turner *et al*., (2002) [17]. 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.

Table 1: Physico-chemical characteristics, acid phosphatase and alkaline phosphatase activity in vertiols

S. No.	pH 1:2	EC 1:2 (dS m^{-1})	Organic carbon (g kg^{-1})	Available (kg ha^{-1})			Sand (%)	Silt (%)	Clay (%)	CEC [cmol (p') kg^{-1}]	Acid Phosphatase*	Alkaline Phosphatase*	Soil Texture
				N	P	K							
1.	8.2	0.20	4.9	168	36	562	16	34	50	42.2	69.3	108.4	Clay
2.	8.1	0.13	3.8	152	21	672	14	33	53	44.4	96.6	95.7	Clay
3.	8.1	0.19	7.8	160	39	474	29	26	45	36.8	104.5	210.3	Clay
4.	8.1	0.15	6.5	167	33	393	32	21	47	39.6	72.1	182.4	Clay
5.	8.6	0.20	4.1	225	15	266	38	17	45	37.6	43.7	210.4	Clay
6.	8.2	0.36	3.7	149	17	309	44	14	42	34.4	34.9	194.2	Clay
7.	8.2	0.16	6.3	243	41	513	40	20	40	32.5	102.5	260.2	Clay loam
8.	8.7	0.33	7.4	126	28	447	32	22	46	39.5	86.4	113.5	Clay
9.	8.6	0.36	4.0	123	20	413	22	21	57	58.7	83.5	192.5	Clay
10.	7.6	0.09	3.9	140	17	159	27	14	59	47.4	74.3	191.8	Clay loam
11.	8.3	0.23	4.3	173	22	707	42	21	37	29.6	89.8	248.8	Clay
12.	8.5	0.16	4.5	154	24	379	42	21	37	31.0	27.3	130.7	Clay loam
13.	8.2	0.27	3.4	135	18	291	12	24	64	57.7	38.8	129.1	Clay
14.	8.4	0.22	4.1	192	20	470	34	13	53	53.3	71.3	130.3	Clay
15.	8.4	0.15	2.9	198	14	271	32	28	40	32.9	49.3	114.6	Clay
16.	8.2	0.11	4.5	233	21	732	32	21	47	39.2	51.7	197.5	Clay
17.	8.7	0.16	6.7	267	42	544	36	15	49	41.6	65.7	400.9	Clay
18.	8.3	0.19	7.4	169	48	407	30	28	42	37.5	108.1	498.7	Clay
19.	8.4	0.32	5.9	145	30	294	31	22	47	38.3	129.3	379.8	Clay
20.	7.9	0.1	4.9	135	25	352	38	21	41	35.3	110.5	200.4	Clay loam
21.	8.4	0.22	4.0	130	20	160	37	23	40	31.3	71.5	90.2	Clay loam
22.	8.2	0.06	4.2	143	23	172	30	25	45	36.8	39.8	91.2	Clay
Min.	7.6	0.06	2.9	123	14	159	12	13	37	29.6	27.3	90.2	
Max.	8.7	0.36	7.8	267	48	732	44	34	64	58.7	129.3	498.7	
Average	8.3	0.2	5.0	171.5	26.5	411.6	31.1	22.1	47.0	40.2	74.1	206.7	

Conclusion

From the results, it can be concluded that in case of vertisols, that there was a significant and positive correlation of acid phosphatase and alkaline phosphate activity with the organic carbon which might be due to fact that the organic matter is the seat of microbial population and activity.

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