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Effect of soil properties on acid phosphatase and alkaline phosphatase activity in alfisols 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. To study the distribution of acid and alkaline phosphatases and influence on physico-chemical properties on soil enzymes, twenty-two soil samples belong to alfisols were collected from different parts 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 16.9 to 268.4 with an average value of 67.3. Similarly, the alkaline phosphatase varied from 16.3 to 185.2 with a mean value of 62.9 µg of 4-nitrophenol g^{-1} soil h^{-1}. The pH ranged from 5.5 to 8.4, electrical conductivity from 0.002 to 0.20 dSm^{-1} and organic carbon from 1.4 to 6.2 g kg^{-1}. The available Nitrogen varied from 118 to 263 kg ha^{-1}. The available P status in the soils varied from 12 to 65 kg ha^{-1}. The range of available K ranged from 81 to 289 kg ha^{-1}. Acid phosphatase was significantly and positively correlated with organic carbon (r = 0.60**) and available P (r = 0.55**). Similarly, Alkaline phosphatase was significantly and positively correlated with organic carbon (r = 0.63**) and available P (r = 0.53**). 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
The acid phosphatase and alkaline phosphatases 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 buildup of these enzymes as well as their activity depends to a great extent 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 phosphatase and alkaline phosphatase in alfisols of Andhra Pradesh and to find out their correlation with soil properties.

Material and Methods
Twenty-two alfisols with widely varying physic-chemical properties collected from different parts of Andhra Pradesh were used for the study. These soils samples were analyzed for their different soil properties viz. physical, physico-chemical and chemical properties. The pH of soil was determined in 1:2.5 soil-water ratio as described by Jackson (1973) [7] using a digital combined glass electrode pH meter (model DI-707). Electrical Conductivity (EC) was determined in 1:2.5 ratio of soil to water extract as detailed by Jackson (1973) [7] 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) [16] method and as described by Jackson (1973) [7]. 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 with the help of international triangle (Singh, 1980) [10]. The available nitrogen (kg ha^{-1}) was determined by Macrokjeldhal distillation method using alkaline potassium permanganate as described by Subbaiah and Asija (1956) [12]. The available phosphorus (kg ha^{-1}) was determined by Olsen'smethod (1954) [9]. The intensity of blue colour developed by L-ascorbic acid was measured by using spectrophotometer at 420 nm.

The available potassium (kg ha^{-1}) was estimated by using neutral normal ammonium acetate extractant (Jackson, 1967) [6] by using Elico flame photo meter. The acid phosphatase activity in alfisols was analyzed by the procedure of Tabatabai and Bremner (1969) [13] and alkaline phosphatase by Eivaji and Tabatabai (1977) [4] 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 1Nsodiumhydroxideandthe 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 of the solution was adjusted to 11with 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 (0.025 M): 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 a refrigerator.

Calcium chloride (0.5 M): This was prepared by dissolving 73.5 g of $CaCl_2.2H_2O$ in distilled water and made up to 1 litre.

Sodium hydroxide (0.5 M): 20 g 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 µg 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 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 11.0 (for assay of alkaline phosphatase) was added followed by the 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 0.5 M $CaCl_2$ was added followed by addition of 4 ml of 0.5 MNaOH 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 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 0.5 M $CaCl_2$ and 4 ml of 0.5 MNaOH. Corrections were made for control/ blank values.

Results and Discussion

The physic-chemical properties of alfisols are presented in Table 1. The pH of the soils varied from 5.5 to 8.4 with a mean of 7.2. The EC of soils has a mean of 0.10 dS/m. The organic carbon values varied from 1.4 to 6.2 with a mean value of 3.2 g kg^{-1}. The available N content varied from 118 to 267 with a mean value of 170.7 kg/ha, the available P ranged from 12 to 65 with a mean of 27.6 kg ha^{-1} and the available K (kg ha^{-1}) varied from 81 to 289 with a mean of 184.0. The CEC of the soils varied from 5.3 to 14.5 with a mean of 9.7 cmol (P+)/kg soil. In general, the texture of the soils varied from sand to sandy loam.

Table 1: Physico-Chemical characteristics, acid phosphatase and alkaline phosphatase activity in alfisols

S.No.	pH 1:2	EC 1:2 (dS m^{-1})	Organic carbon (g kg^{-1})	Available			Sand (%)	Silt (%)	Clay (%)	CEC [cmol (p^+) kg^{-1}]	Acid Phosphatase*	Alkaline Phosphatase*	Soil Texture
				N	P	K							
					kg ha^{-1}								
1	7.6	0.08	1.8	144	12	127	83	4	13	9.2	46.8	24.7	LS
2	5.5	0.02	1.4	157	15	116	87	3	10	7.7	27.8	38.9	S
3	8.4	0.14	3.6	161	22	145	72	11	17	12.4	22.4	119.3	LS
4	6.2	0.02	1.9	118	17	210	77	6	17	11.5	67.5	53.2	LS
5	8.0	0.08	2.8	122	19	140	85	3	12	8.1	17.3	29.5	LS
6	7.2	0.15	3.0	141	23	227	83	2	15	9.4	38.4	56.4	SL
7	6.8	0.03	5.5	227	50	192	81	3	16	12.3	268.4	104.1	SL
8	7.7	0.05	2.3	118	18	255	78	5	17	11.1	36.8	19.7	SL
9	8.0	0.06	4.0	129	31	118	82	5	13	9.3	35.1	97.1	LS
10	8.1	0.05	3.2	263	16	269	83	3	14	9.9	16.9	32.8	SL
11	6.5	0.04	2.9	231	32	235	76	7	17	11.4	98.8	60.5	SL
12	6.4	0.03	4.1	212	37	171	83	2	15	9.7	157.1	65.7	SL
13	7.4	0.02	4.7	243	42	289	76	8	16	12.2	78.6	86.5	SL
14	7.2	0.06	6.2	267	65	262	73	9	17	11.3	71.2	70.4	SL
15	6.4	0.02	2.6	121	21	183	84	4	12	7.3	35.6	16.3	LS
16	7.5	0.08	2.4	190	23	221	85	4	11	7.3	60.8	65.9	LS
17	8.1	0.11	4.7	133	39	180	79	9	12	7.9	40.5	185.2	LS
18	6.7	0.04	1.6	155	18	81	90	3	7	5.3	37.1	24.7	S
19	7.1	0.03	2.7	127	25	192	76	6	18	14.5	24.4	20.5	SL
20	7.6	0.20	3.2	138	23	105	79	10	11	9.2	18.1	28.9	LS
21	7.2	0.11	2.5	185	20	153	79	7	14	8.8	51.1	46.4	LS

22	7.7	0.09	2.9	129	17	174	85	4	11	7.5	79.9	60.3	LS
Min	5.5	0.02	1.4	118	12	81	72	2	7	5.3	16.9	16.3	
Max	8.4	0.2	6.2	267	65	289	90	11	18	14.5	268.4	185.2	
Average	7.2	0.1	3.2	170.7	27.6	184.0	80.8	5.5	13.8	9.7	67.3	62.9	

The acid phosphatase and alkaline phosphatase activity in alfisols are presented in Table 2. The acid phosphatase varied from 16.9 to 268.4 with a mean of 67.3 µg of 4-nitrophenol g^{-1} soil h^{-1}. The alkaline phosphatase varied from 16.3 to 185.2 with a mean value of 62.9 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 and is presented in Table 2.

The acid phosphatase activity was significantly and positively correlated with organic carbon (0.60**) and available phosphorus (0.55**) in alfisols. 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 (0.63**) and available phosphorus (0.53**) in alfisols 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) [8, 11] Chhonkar and Tarafdar (1994) [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) [8] observed significant positive correlation between phosphatase activity and organic matter content in soil. While Harrison, (1983) [5] reported a significant positive relationship between phosphatase activity and organic carbon content in woodland soils. Tarafdar *et al* (1981) [14] observed a significant and positive correlation of alkaline phosphatase with soil organic carbon in jute growing soils of West Bengal.

Table 2: Correlation of phosphatase activities with soil properties

Soil properties	Acid phosphatase	Alkaline phosphatase
Organic carbon	0.60**	0.63**
pH	-0.27	-0.32
Clay	0.29	0.14
CEC	0.26	0.13
Available P	0.55**	0.53**
Available N	0.38	0.16
Available K	0.19	0.21

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. The results of correlations and linear equations between phosphatase activity and soil characteristics was also 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. Zibilske and Bradford, (2003) [17] have found significant correlation between phosphatase activity, extractable P and dissolvable organic carbon. Similar findings were also given by Turner *et al*., (2002) [15]. 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.

Conclusion

From the results, it can be concluded acid phosphatase and alkaline phosphatases in alfisols showed a significant and positive correlation with the organic carbon which could be due to the fact that the organic matter is the seat of microbial population and activity.

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