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Effect of Buffer pH on Soil Phosphomonoesterase Activity in Vertisols of Andhra Pradesh, India

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A B S T R A C T

The effect of buffer pH on soil phosphomonoesterase activity was carried out in laboratory conditions in vertisols using modified universal buffer with a pH range from 3-12. The pH of the MUB was adjusted to respective pH levels using 0.1 N HC1 or 0.1 N NaOH respectively. The substrate p⁻ nitrophenyl phosphate of 0.025 M concentration was prepared in MUB of corresponding pH and analyzed for phosphomonoesterase activity. The soils were also analyzed for the physicochemical properties like pH, EC, available nutrients, texture and organic carbon. The results indicated that there was significant increase in the activity of phosphomonoesterase in the pH from 3 to 11.0 and later a steady decrease at pH 12. Thus all the vertisols exhibited a single peak for phosphatase activity at pH 11.0. On the other hand, two peaks of phosphomonoesterase activity at pH 6.5 and pH 11.0 was registered by the soil S-2, S-3, S-4, S-5 and S-6. The mean activity of phosphomonoesterase at pH 11.0 was found to be 231.3 µg of 4-nitrophenol released g^{-1}soil $h^{-1.}$

Introduction

Soil enzymes are important for catalyzing 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). All the enzymes are protein in nature and exhibit peak activity over a narrow pH range. This depends on the nature of the enzyme and is due to the dissociation and protonation of acid and amino groups particularly connected with the binding site. Studies on the effects of pH on soil enzymes is important because exposure to extreme pH values may irreversibly inactivate the enzymes that play an essential role in nutrition (N, C, P and S) tranformations and humus formation.

The influence of pH on enzyme activity can be distinguished experimentally by first incubating the soils at an indicated pH for a particular period atleast as long as the assay

time and measuring the enzyme activity. In agricultural soils, the buildup of these enzymes as well as their activity depends mostly on the soil properties, crop plants and farming systems. The present investigation was undertaken to study the effect of buffer pH on soil phosphomonoesterase in vertisols of Andhra Pradesh.

Materials and Methods

Ten 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) 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) 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) method and as described by Jackson (1973). 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). The available nitrogen (kg ha^{-1}) was determined by Macrokjeldhal distillation method using alkaline potassium permanganate as described by Subbaiah and Asija (1956). The available phosphorus (kg ha^{-1}) was determined by Olsen's method (1954). 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) by using Elico flame photo meter. The assay of phosphomonoesterase was carried out by the procedure described by Tabatabai and Bremner (1969) and Eivaji and Tabatabai (1977).

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.

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 73.5g of $CaCl_2.2H_2O$ in distilled water and made up to 1 litre.

Sodium hydroxide (0.5 M)

20 g of sodiumhydroxide was dissolved in 700 ml of distilled water and diluted to 1 litre with water.

Standard p-nitrophenol solution

Primary stock solution of 1000 $\mu g ml^{-1}$ 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 standards of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg ml^{-1} were prepared from 20µgml^{-1} stock and the absorbance of these standards were recorded at 420 nm in spectrophotometer. This was used for the standard curve.

Procedure

The effect of pH on soil phosphomonoesterase activity was carried out in quadruplicates in selected vetisols using modified universal buffer with a pH range from 3-12. The pH of the MUB was adjusted to respective pH levels using 0.1 N HC1 or 0.1 N NaOH respectively. The substrate p–nitrophenyl phosphate of 0.025 M concentration was prepared in MUB of corresponding pH.

One gram soil was taken in a glass tube, to this 4 ml of MUB (of respective pH) and 1 ml of substrate (of same pH) was added and incubated at 37+0.5^{o}C for 1 hour 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^{o}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 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 phosphomonoesterases 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 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH. 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 8.1 to 8.7 with a mean of 8.3. The EC of soils has a mean of 0.24 dS/m. The organic carbon values varied from 3.7 to 8.6 with a mean value of 5.9 g ha^{-1}. The available N varied from 123 to 243 with a mean value of 181 kg ha^{-1}, the available P ranged from 16 to 47 with a mean of 30 kg ha^{-1}. and the available K varied from 393 to 709 with a mean of 525 kg ha^{-1}. The CEC of the soils varied from 30 to 48 with a mean of 39 C mol (P+) kg soil^{-1}. In general, the texture of the soils varied from Clay loam to Clay.

The effect of buffer pH on phosphomonoesterase in vertisols is presented in Table 2. In soils, S-1, S-7 to S-10, there was a significant increase in the activity of phosphomonoesterase (depicted in Fig.1) in the pH from 3 to 11.0 and a decrease was observed at pH 12.0. Thus only one peak at pH 11 was present. On the other hand, two peaks of phosphomonoesterase activity at pH 6.5 and pH 11.0 were registered by the soil S-2, S-3, S-4, S-5 and S-6(depicted in Fig 2). The mean activity of phosphomonoesterase at pH 6.5 was 100.4 µg of 4-nitrophenol g $^{-1}$ soil h^{-1} at pH 11.0 was 231.3 µg 4-nitrophenol g^{-1} soil h^{-1}.

The results of the variation in phosphomonesterase activity with pH of soil is significant, and most of the vertisols had a dominant peak at pH 11.0. Some of the vertisols however showed a peak at 6.5 as well as at pH 11.0, though the latter was always more pronounced. Similar results for soil phosphomonesterases were reported by workers such as Eivazi and Tabatabai (1977).

Eivazi and Tabatabai (1977) studied the effect of buffer pH ranging from 4 to 12 on phosphatase activity in some acid and alkaline

phosphatase (buffer pH 6.5) is pre-dominant in acid soils and that alkaline phosphatase in alkaline soils. Herbien and Neal (1990) studied the influence of some pH on the phosphomonoesterase activity in the soils planted to Oak (forest), grass (grassland) and corn (agriculture), respectively. In the forest soil, only acid phosphomonoesterase was detected whose pH optima was maximal at the measured soil pH of 4.9. A neutral phosphomonoesterase with a broad pH optima ranging from 4.6 to 7.0 was found in the grass-land soil of pH 6.6, while both acid and alkaline posphatases with a pH of 4.8 to 11.0 respectively, were found associated with the agricultural soil of pH 7.2. The results of this study indicate that a relationship exists between soil pH and (i) the synthesis and release of phosphatase in soil, (ii) the complexion of organisms producing the enzymes and (iii) phosphatase stability or conformation. They further concluded that the analysis of phosphatase activity at the measured soil pH is necessary to determine the contribution of phosphatase enzymes to the cycling of phosphorus.

Frankenberger and Johanson (1982) indicated that the variation in pH stability of enzymes was due to different sources contributing to enzyme activity and to adsorption properties of soils themselves. The diversity of vegetation, microorganisms and soil fauna as a source of enzymes could be responsible for the difference in pH stability of enzyme activities.

When soil enzymes are exposed to extreme acid or alkaline conditions the catalytic activity of enzyme protein decreased probably because of the pH effect on the overall three dimensional structure of the protein itself.

Table.1 Physico-chemical characteristics of Vertisols

S.No.	pH 1:2	EC 1:2 (dS m^{-1})	Organic carbon (g kg^{-1})	Available (kg ha^{-1})			CEC [Cmol (p')kg^{-1}]	Sand (%)	Silt (%)	Clay (%)	Soil Texture
				N	P	K					
S-1	8.20	0.20	8.6	158	47	562	42.2	16	34	50	Clay
S-2	8.10	0.13	3.8	188	21	672	44.4	14	33	53	Clay
S-3	8.10	0.19	7.8	216	39	474	36.8	29	26	45	Clay
S-4	8.10	0.15	6.5	167	33	393	39.6	32	21	47	Clay
S-5	8.20	0.16	8.3	243	41	513	32.5	40	20	40	Clay loam
S-6	8.70	0.33	7.4	126	36	447	39.5	32	22	46	Clay
S-7	8.60	0.36	4.0	123	20	413	48.0	22	21	57	Clay
S-8	8.30	0.23	4.3	173	22	709	29.6	42	21	37	Clay loam
S-9	8.10	0.38	3.8	215	16	543	38.4	25	30	45	Clay
S-10	8.40	0.22	3.7	192	20	470	41.0	34	13	53	Clay
Min	**8.70**	**0.38**	**8.6**	**243**	**47**	**709**	**48.0**	**42**	**34**	**57**	
Max	**8.10**	**0.13**	**3.7**	**123**	**16**	**393**	**30.0**	**14**	**13**	**37**	
Average	**8.30**	**0.24**	**5.9**	**181**	**30**	**525**	**39.0**	**29**	**24**	**47**	

Table.2 Effect of buffer pH on soil phosphomonoesterase activity in Vertisols

Buffer pH	(µg of 4-nitrophenol released g^{-1} soil h^{-1})										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**Mean**
3.0	62	62	30	41	25	28	18	61	24	15	36.6
4.0	81	71	48	63	45	47	26	74	33	28	51.5
5.0	97	81	70	87	58	62	35	92	44	39	66.4
6.0	111	91	94	103	69	73	50	111	56	51	80.9
6.5	143	111	105	130	89	95	86	126	68	70	100.4
7.0	167	86	88	99	65	71	70	140	80	86	95.2
8.0	181	108	121	136	81	87	78	157	91	100	114.0
9.0	193	128	169	190	99	102	96	174	104	115	136.9
10.0	209	162	221	253	114	121	107	191	124	132	163.3
11.0	272	218	296	337	200	190	182	241	180	197	231.3
12.0	175	139	177	203	92	82	74	139	78	69	122.8
Mean	154.7	114.3	129.0	149.3	85.2	87.0	72.8	136.9	80.2	82.0	
Analysis of variance			F test		S.Ed.		CD at 5%				
Soils			**		1.3		2.5				
Buffer pH levels			**		1.4		2.7				
Soils x Buffer pH levels			**		4.3		8.4				

Figure.1 Effect of buffer pH on soil phosphomonoesterase activity in Vertisols (2,3 to 6)

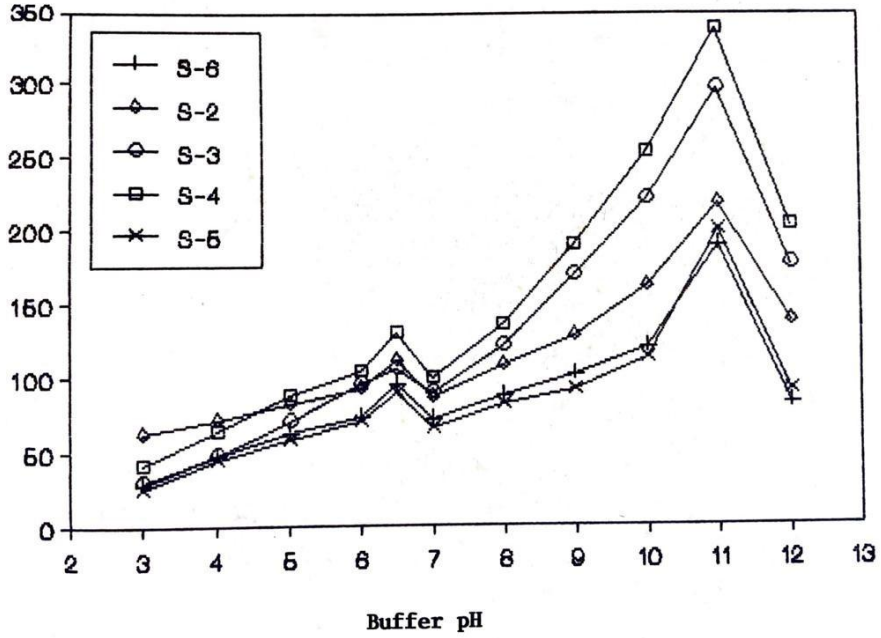
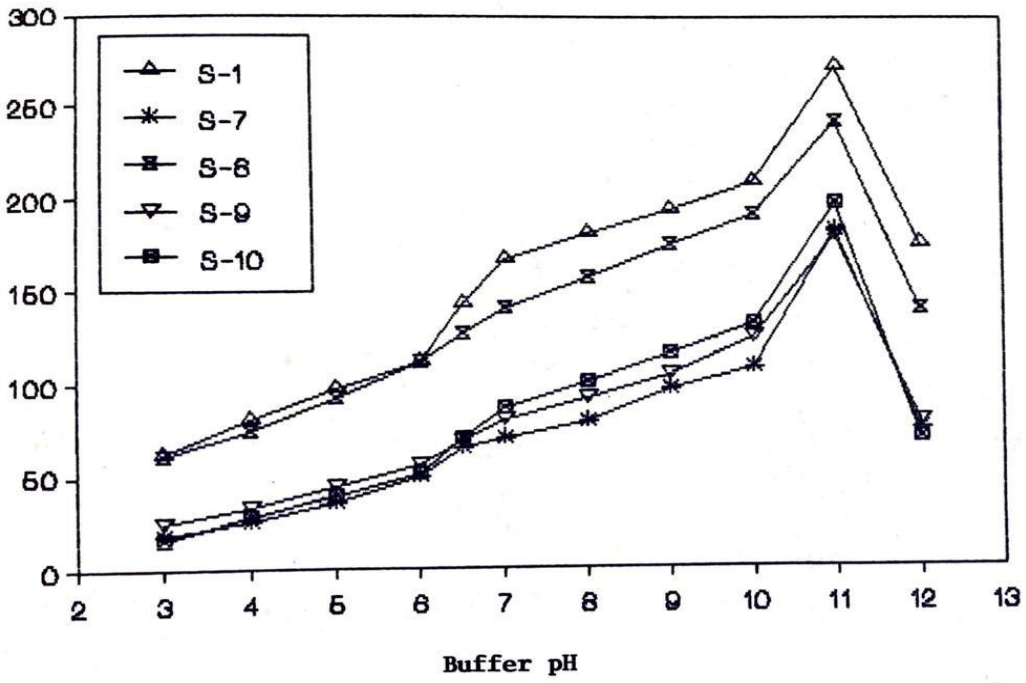


Figure.2 Effect of pH on soil phosphomonoesterase activity in Vertisols (1,7 to 10)



Denaturation results when the ordered structure of a globular protein is altered into a randomly non-functional disordered arrangement of peptide chains. Exposure to a high H^+ ion concentration or OH^- ion concentration tends to disrupt the ionic and hydrogen bonds needed to maintain active conformation of the enzyme resulting in loss of biological activity.

The pH of soil solution exerts a strong control on the enzyme activity because I t influences the conformation of enzyme, its absorption on solid surfaces ionization and the solubility of substrates andco-factors (Turner, 2010; Frankenberger and Johanson, 1982) indicated that the variation inpH stability of enzymes was due to different sources contributing to enzyme activity and to adsorption properties of soils themselves. In a study conducted by Shi *et al.,* (2008) found that soil pH had a negative effect onurease and phosphatase activity but the effect was counter acted by the positive in direct effect of soil organic matter.

The results of the variation in phosphomonoesterase activity with pH of the soil is significant. Most of the vertisols have a dominant peak at pH 11.0 but few soils have showed two peaks at pH 6.5 and pH 11.0. When soil enzymes are exposed to extreme acid and alkaline conditions, the catalytic activity of enzyme protein decreased due to effect of pH on overall three dimensional structure of protein itself. Further, exposure to a high H^+ ion concentration tends to disrupt the ionic and hydrogen bonds needed to maintain active confirmation of the enzyme resulting in loss of biological activity.

References

Bouyoucos, G. J., 1962. Hydrometer method improved for making particle size analyses of soils.

Agronomy.Journal.54:464-465.

Dick, R. P. 1994. Soil enzyme activities as indicator of soil quality. In J. V. Doran, D. C. Coleman, D. F. Bezdicek and V. A. Stewart (eds.) – *Defining Soil Quality for Sustainable Environment*, SoilScience Society of America, American Society of Agriculture, Madison. 107 –124.

Eivaji, F and Tabatabi, M. A. 1977. Phosphatases in soils. Soil Biology and Biochemistry 9: 167-172

Frankenberger, W. T. Jr. and Johanson, J. B. 1982. Effect of pH on enzyme stability in soils. Soil Biology and Biochemistry 14: 433-437.

Herbien, S. A. and Neal, J. L. 1990. Soil pH and phosphatase activity. Communications in Soil Science and Plant Analysis. 21: 439-456

Jackson, M. L., 1973. Soil chemical analysis. *Prentice Hall of India Private Limited*., New Delhi.

Jackson, M. L., 1967. Soil chemical analysis. *Prentice Hall of India*, New Delhi.

Olsen, S. R., Cole, C. V., Watanabe, F. S and Dean, L. A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *Circulation from USDA*, 939.

Shie, Z. J., Lu, Y., Xu, Z. G and Fu, S. L. 2008. Enzyme activities of urban soils under different land use in the Shenzhen city, China. Plant Soil Environment.54(8):341-346.

Singh, D., Chhonkar, P. K. and Pandey R. N. 1980. Soil plant water analysis *In: A Methods Manual*. Indian Agricultural Research Institute, New Delhi.

Subbaiah, B. V., and Asija, G. L. 1956. A rapid procedure for the determination of available nitrogen in soils.*CurrentScience*.25: 259-260.

Tabatabai. M. A. and Bremner, J. M. 1969. Use of p-nitrophenyl phosphate for assay soil phosphatase activity. Soil Biology and Biochemistry 1: 301-307.

Turner, B. L., 2010. Variation in pH optima of hydrolytic enzyme activities in tropical rain forest soils. Applied and Environmental Microbiology.76(19): 6485-6493.

Walakley, A., and Black, C. A. 1934. Estimation of organic carbon by chromic acid titration method.*SoilScience*.37: 29-38.

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