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Investigations on the Effect of Buffer pH on Soil Phosphomonoesterase Activity in Alfisols of Andhra Pradesh, India

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A B S T R A C T

The studies on the effect of buffer pH on soil phosphomonoesterase activity was carried out in laboratory conditions in alfisols 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.IN 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 6.5 and later a steady decrease till pH 12. Thus all the alfisols exhibited a single peak of phosphomonoesterase activity at pH 6.5. The mean activity of phosphomonoesterase at pH 6.5 was found to be 132 µg of 4-nitrophenol released g^{-1} soil h^{-1}. The soils S-6, S-8 and S-10 recorded significantly higher activity of phosphomonoesterase at all the pH levels used in the study.

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). 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). 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 alfisols of Andhra Pradesh.

Materials and Methods

Ten alfisols 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 ($mg\ kg^{-1}$) 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 to 1 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 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 (0.5 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 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 standards 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 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 alfisols 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 4ml 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 0.5M $CaCl_2$ and 4 ml of 0.5 M NaOH. Corrections were made for control/ blank values

Results and Discussion

The physic-chemical properties of alfisols are presented in Table 1. The pH of the soils varied from 5.5 to 7.4 with a mean of 6.48. The EC of soils has a mean of 0.06 dS m^{-1}. The organic carbon values varied from 1.4 to 6.2 with a mean value of 3.4 g kg^{-1}. The available N content in soils varied from 118 to 267 with a mean value of 196 kg ha^{-1}, the available P ranged from 15 to 65 with a mean of 33 kg ha^{-1} and the available K varied from 116 to 289 with a mean of 215 kg ha^{-1}. The CEC of the soils varied from 5.3 to 11.8 with a mean of 9.4 Cmol (P+) kg $soil^{-1}$. In general, the texture of the soils varied from sand to sandy loam.

The effect of buffer pH on phosphomonoesterase in alfisols is presented in Table 2 there was significant increase in the activity of phosphomonoesterase In the pH from 3 to 6.5 and then a steady decrease till pH 12. Thus all the alfisols exhibited a single peak for phosphatase activity at pH 6.5. The mean activity of phosphomonoesterase at pH 6.5 was found to be 132 µg of 4-nitrophenol released g $^{-1}$ soil h $^{-1}$.The soils S-6, S-8 and S-10 recorded significantly higher activity of phosphomonoesterase at all the pH levels used in the study.

The results of the variation in phosphomonesterase activity with pH of soil is significant, and all the alfisols exhibited a single peal kat pH 6.5. Similar results for soil phosphomonesterases was reported by workers 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.

Table.1 Physico-chemical characteristics of Alfisols

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	5.6	0.14	1.7	168	16	226	7.4	85	4	11	Loamy sand
S-2	6.1	0.07	1.9	206	19	242	5.3	90	3	7	Sand
S-3	5.5	0.02	1.8	157	15	116	7.7	87	3	10	Sand
S-4	6.2	0.02	1.4	118	17	210	11.5	77	6	17	Loamy sand
S-5	7.2	0.15	3.0	141	23	227	9.4	83	3	14	Sandy loam
S-6	6.8	0.03	5.5	227	50	192	10.8	81	3	16	Sandy loam
S-7	6.5	0.04	2.9	231	32	235	11.4	76	7	17	Sandy loam
S-8	6.4	0.03	4.1	212	37	171	9.7	83	2	15	Sandy loam
S-9	7.4	0.02	4.7	243	42	289	10.2	76	8	16	Sandy loam
S-10	7.2	0.06	6.2	267	65	262	11.8	73	9	18	Sandy loam
Min	**5.50**	**0.02**	**1.4**	**118**	**15**	**116**	**5.3**	**73**	**2.0**	**7.0**	
Max	**7.40**	**0.15**	**6.2**	**267**	**65**	**289**	**11.8**	**90**	**9.0**	**18.0**	
Average	**6.48**	**0.06**	**3.4**	**196**	**33**	**215**	**9.4**	**81**	**4.9**	**13.8**	

Figure.1 Effect of buffer pH on Soil Phosphomonoesterase activity in Alfisols (1 to 5)

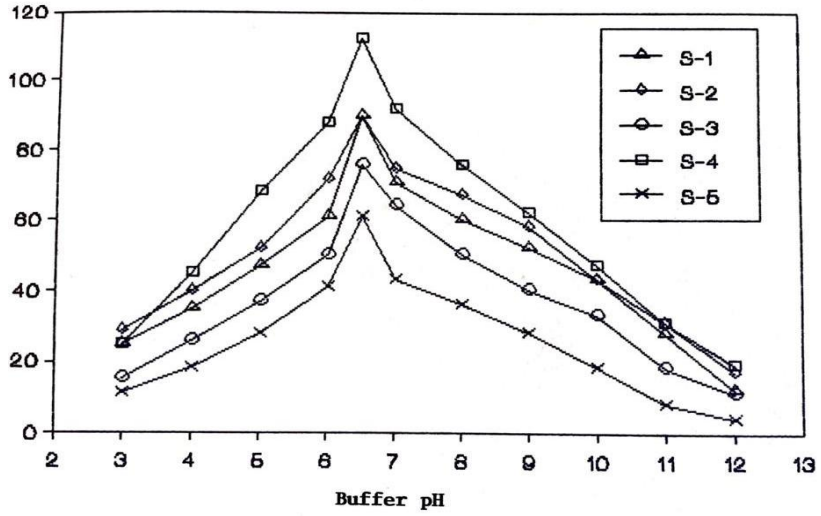
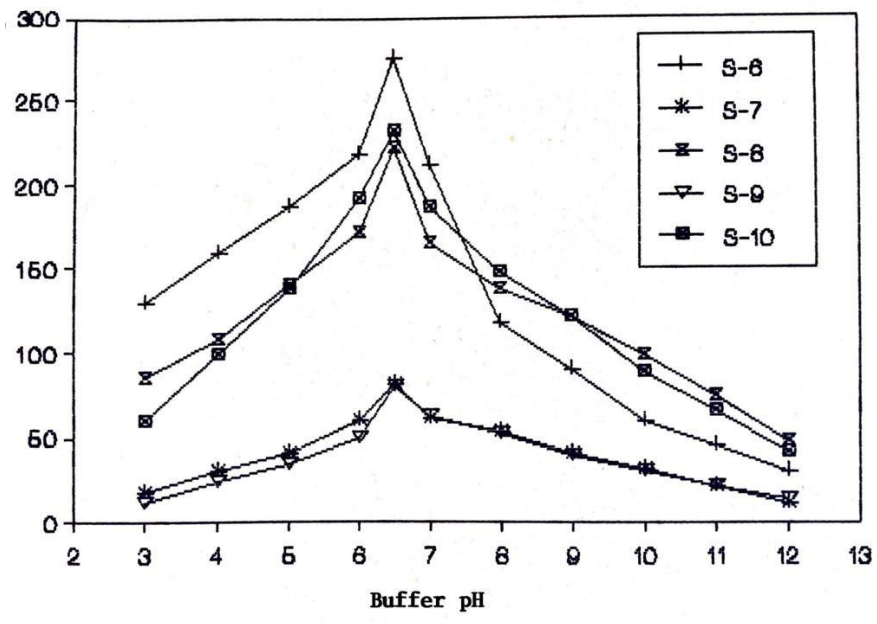


Table.2 Effect of buffer pH on soil phosphomonoesterase activity in Alfisols

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	25	29	15	25	11	129	18	85	12	60	40.9
4.0	35	40	26	45	18	159	30	108	24	99	58.4
5.0	47	52	37	68	28	187	41	140	34	138	77.2
6.0	61	72	50	88	41	218	60	172	50	192	100.4
6.5	90	90	76	112	61	275	81	222	80	233	132.0
7.0	71	75	64	92	43	212	61	165	62	187	103.2
8.0	60	67	50	76	36	117	53	138	52	148	79.7
9.0	52	58	40	62	28	45	40	121	39	121	70.6
10.0	43	43	33	47	18	117	32	99	30	89	55.1
11.0	28	31	18	31	8	102	21	75	21	66	40.1
12.0	12	17	11	19	4	85	11	48	14	42	26.3
Mean	52.2	52.2	38.2	60.5	26.9	158.7	40.7	124.8	38.0	125.0	
Analysis of variance			**F test**		**S.Ed.**		**CD at 5%**				
Soils			**		**0.7**		**1.5**				
Buffer pH levels			**		**0.8**		**1.6**				
Soils x Buffer pH levels			**		**2.6**		**5.2**				

Figure.2 Effect of pH on Soil Phosphomonoesterase activity in Alfisols (6 to 10)



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. 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 it 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 on urease 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. All the alfiols have a a dominant peak at pH 6.5. The soils S-6, S-8 and S-10 recorded significantly higher activity of phosphomonoesterase at all the pH levels used in the study. 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.

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