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S Balo
Department of Soil Science and
Agricultural Chemistry, Uttar
Banga Krishi Viswavidyalaya,
Pundibari, Cooch Behar,
West Bengal, India

A Debnath
Department of Agricultural
Chemistry and Soil Science,
Bidhan Chandra Krishi
Viswavidyalaya, Mohanpur,
Nadia, West Bengal, India

A Sen
Department of Agricultural
Chemistry and Soil Science,
Bidhan Chandra Krishi
Viswavidyalaya, Mohanpur,
Nadia, West Bengal, India

Corresponding Author
S Balo
Department of Soil Science and
Agricultural Chemistry, Uttar
Banga Krishi Viswavidyalaya,
Pundibari, Cooch Behar,
West Bengal, India

Study on the potentiality and limitation of Inositol hexaphosphate in phosphorous nutrition to Bengal Gram

S Balo, A Debnath and A Sen

Abstract

Inositol hexaphosphate or phytic acid is an important component of soil organic Phosphorous (P). The behaviour of phytic acid or phytate in soil was studied in terms of its adsorption desorption. This was compared with its sorption in goethite and nutrient free sand. To test the availability of P of phytate, Bengal gram was grown in pots using soil and sand as media of growth. Plants were applied with various amounts of phytate and /or inorganic soluble sources of P, compared dry matter weight and P uptake in crop as affected by media of growth and source of P. Phytate sorption was higher in the soil of Jhargram than Chuapara Tea Garden, being two soils used in this study. This was caused as a result of prior enrichment of soil with inorganic and organic P in Chuapara. Similarly, goethite adsorbed much higher amount of phytate, similar to soils, than that of sand. In terms of desorption of the sorbed phytate the soils and the goethite were similar in trend showing considerable hysteresis thereby high buffering capacity. Sand had no or very insignificant site for phytate sorption could not retain phytate during desorption so, exhibiting little hysteresis. The dry-matter weight and P uptake of Bengal gram was noted highest with 100 mg P kg⁻¹ applied in inorganic soluble form in the soil. This was followed by addition of phytate P in sand at corresponding rate of application of P. At lower rates of P (25 mg kg⁻¹) inorganic and organic P applied in sand resulted higher dry matter weight and P uptake than the values in soil. This revealed that phytate P could be utilized by plants provided that it remains free in solution. The finding from the present study suggest that organic P in the form phytate is a good source of P and sometimes better than the inorganic soluble forms. Phytate is strongly adsorbed on to the binding sites of soil particles rendering it unavailable in soil solution and thereby hydrolysis by the enzymes of microbial or plant root origin. Further research should be aimed at finding ways and means to hydrolyse P of phytate that is adsorbed on to soil components.

Keywords: Phytate, goethite, sand, Bengal gram, sorption, desorption

Introduction

Phosphorous is essential for all forms of life and is equally important for its contribution in aiding. Phosphorous is a non renewable resource and at the current rate of consumption of phosphate rock the world is able to produce fertilizer P for about 100 years with the present relative cost of production (Heffer *et al.*, 2006) [9]. However, with the projected increase in annual consumption of 20 Mt by the year 2030, as was estimated in the year 2000 (Vance *et al.*, 2003) [15], the actual reason might last for even less than 100 years. Thus, the crop growth and its yield are likely to suffer adversely unless soil is endowed with adequate native supply of plant available P. This will severely affect the resource poor farmers of the tropics and the sub tropics. Therefore, there is a need to manage P in crop nutrition for sustainable yields for which a better understanding of the behaviour of P in soil is important; the other important aspect is the development of P efficient cultivars of crops which will use soil and fertilizer P more efficiently. Organic forms of P can be a substantial reserve of plant available form of P. Phosphomonoesters and diesters comprise at least 25% of total P in soil while the inositol phosphorous can represent 20-25% of total organic P soil. Among the inositol phosphate synthesised by plants inositol hexaphosphate monoester is abundant which contains six phosphate groups in inositol ring. Inositol hexaphosphate is also named as phytic acid. It has 12 replaceable protons and the molecule is negatively charged in the pH range of 5.0 to 7.0 of common soils, and because it is negatively charged phytic acid is strongly adsorbed on to the surface of Fe and Al oxides present in soils, therefore, the adsorption is pH dependent similar to other anions. Hydrolysis of phosphate groups of phytic acid can render P available to plants. Inositol hexaphosphate is at least equal to potassium orthophosphate (KH₂PO₄) as a source of

P for the growth of lupins in sand, but a much poorer source in soil (Adam and Plate, 1992) [1]. Consequently many have tried to improve access to such phosphate by breeding plants to ascertain increased ability to excrete phosphatase. However, those have been generally unsuccessful. Since IP_6 is adsorbed strongly onto the surface of soil particles, Barrow (2015) envisaged that the link to the surface via the phosphate moieties is thus protected from attack by phytases. There are two consequences to follow from this concept. One is that the effectiveness of IP_6 , as a source of phosphate to the plants, will depend upon the proportion in solution and phytase will only be effective when there is a large proportion of IP_6 in solutions. The other consequence is that with an ability to desorb adsorbed IP_6 should have better access to it. The higher retention IP_6 as a consequence of its stronger adsorption in soil than orthophosphate anions and limited access of enzyme phytase to IP_6 in have resulted accumulation of this fraction of organic P in soil. Thus, in order to investigate the retention behaviour of IP_6 in soil and the ease of access of phosphate group of IP_6 to plants, the present work was undertaken to study the adsorption desorption of phytic acid in soil and in pure system to evaluate its retention ability buffering power and to grow plants both on soil and sand in pools to compare the effectiveness of IP_6 with easily plant available orthophosphate form of P, thereby to understand the mechanisms of phosphate release from IP_6 molecule.

Materials and Methods

To study phytic acid sorption and desorption of soils experiments were conducted in laboratory. Surface (0-20 cm) soil sample were collected from Jhargram district (Alfisol) and Chuapara of Jalpaiguri district (Inceptisol). These soils were air dried, thoroughly mixed and ground to pass through a 2 mm sieve. The pH of soils was determined by using soil suspensions in water in the ratio of (1:2.5) (Jackson, 1973) [10] and a glass electrode pH meter. The water holding capacity of soil was determined by Keen Rackzowski box as described by Baruah and Barthakur (1997) [6]. The organic carbon in the soil was estimated by the method of Walkley and Black (1934) [16]. The Colwell method as modified by Kuo (1996) [11] was used for analysis of phosphorus in soil. The phytic acid sorption-desorption and pot culture studies were conducted. Further, the Phosphorous analysis in plant sample was done as per the methods described by Singh *et al.*, (2005) [13] and the vanadomolybdate method.

The Freundlich equation, modified by adding an intercept term (q) (Barrow, 2008) was fitted to the data:

$$S = ac^b - q \quad (1)$$

Where S = sorption of phytate in $mg\ kg^{-1}$, C= the observed solution concentration in $mg\ phytate\ L^{-1}$, and a, b, q are parameters. The intercept (q) is formally the value for desorption at zero solution concentration of phytate.

For the sorption step, sorption was calculated from the observed change in concentration.

$$S = (c_i - c) S_r \quad (2)$$

Where, S_{sr} = the solution : soil ratio, c_i = the initial solution concentration before mixing the soil. For the desorption step, sorption was calculated from

$$S_d = S - c S_{sr} \quad (3)$$

Where S_d = indicates the amount of phytate retained by soil after the desorption step. The data both sorption and desorption were fitted simultaneously and there were, therefore, five parameter. These may be represented as: a_s , b , q_s , a_d and q_d where subscripts s and d refer to the sorption and desorption steps. Because sorption and desorption are calculated from the observed solution concentration, the two variables of equations (1) and (3) are not independent. In order to fit Equations (1) or (3), they were regarded as simultaneous with equation (2). For a given set of parameters, the intersection of the two equations was found. This gives the predicted values for the observed variable (solution concentration) for given values of the experimental variables (initial solution concentrations and solution:soil ratio) (Barrow, 2008) [3]. A Simplex procedure was used to find the set of parameters that minimized the sums of squares for the difference between the logs of observed and predicted concentrations.

For curve fitting, the Sigma Plot (version 10) was used and for all statistical calculations regarding plant response were carried out using the statistical software SPSS (version 16).

Results and Discussion

A few properties of the soils under study are presented in Table 1. Both the soils are belonging to two acidic zones of West Bengal. The soil of Jhargram belongs to Red and Laterite zone while that of Chuapara is from a tea Garden in the district of Jalpaiguri. Soils are strongly acidic in nature. The organic carbon content was very low to the Jhargram (0.39%) but high in the Chuapara (0.91%) soil. The water holding capacity was higher in the Chuapara than the Jhargram soil which seems to correlate with the organic matter content of the soils. The values of Colwell P in this soils indicated that the highly weathered soil of Jhargram is very poor in P status than the Chuapara soil, the latter being receiving soluble and sparingly soluble phosphate rock every year. Two pure systems *viz.*, goethite [$FeO(OH)$] and nutrient as well as organic matter free sand were considered in this study for laboratory experiments being representative of two pure systems.

Figures 1 and 2 show the sorption- desorption curves of phytate in the soils and pure systems. Figure 3a compares the sorption between soils and 3b compares that between goethite and sand. The curves are generated using Freundlich model modified by incorporating an intercept term "q" (Barrow, 2008) [3] which is formally the value for sorption at 0 solution concentration of phosphate.

Except for sand, in all two soils and goethite, the desorption curves were found highly differing from the sorption curves and that hysteresis occurred. However, for sand the desorption curves were very close to sorption curve, thus showing little hysteresis and hence low buffering power. The hysteresis ratio, as indicated in the work of Barrow and Debnath (2014) [4], was calculated from the ratio of the term "a" for desorption and sorption.

Table 2 summarizes the parameter of sorption desorption of phytate in the soils and pure media. The soils and goethite having variable charge surfaces exhibited higher values of slope of the curves than that of sand as measured at $20\ mg\ kg^{-1}$ solution concentration. This is an estimate of the ability of the medium to retain phytate from solution, Goethite has been recorded highest value of hysteresis amongst all followed by Jhargram, and sand having the least value. The extent of hysteresis may be regarded as the buffering power of the soils

/medium. The less the value of hysteresis; the less is the buffering power.

Figure 4 depicts the relationship between the hysteresis ratio and “null point” values. Null point is the concentration of phytate in solution when there is no sorption or desorption of phytate. The null point concentration decreases with increase in the value of hysteresis ratio indicating a low availability of phytate in solution for enzymatic action. The dry matter weights of Bengal gram for 4 plants under different treatments of P source and amounts added have been presented in figure 5 and that the total P uptakes have been shown in figure 6. These figures compare the source and medium of growth at each level of application of inorganic and phytate form of the P while figure 6 describes the overall performance of the source of P in medium irrespective of rate of application of P. These figures revealed that at low rate of

P application, dry matter weight and P uptake are significantly higher in sand irrespective of P source. At higher level, soil application of Pi recorded highest values of yield attributes followed by sand application of Pi. Phytate application in sand was found significantly better than its application in soil. Figure 7 summarizes the response curve arising out of dry matter weight and P uptake in plants against different forms and rates of Phosphorous.

Table 1: Parameters of studied soil

Soil	Jhargram	Chuapara
pH	4.45	4.86
Organic carbon	0.51%	0.90%
Water holding capacity	33.1%	4.1%
Colwell P	15.590 mg kg ⁻¹	109.826 mg kg ⁻¹

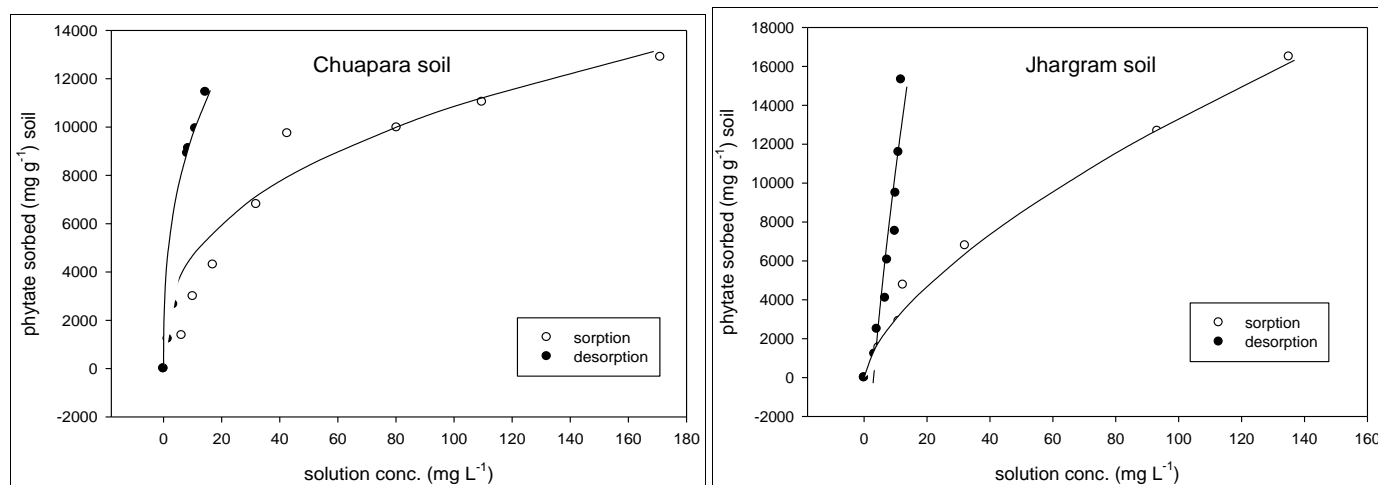


Fig 1: Sorption Desorption curve of Jhargram and Chuapara

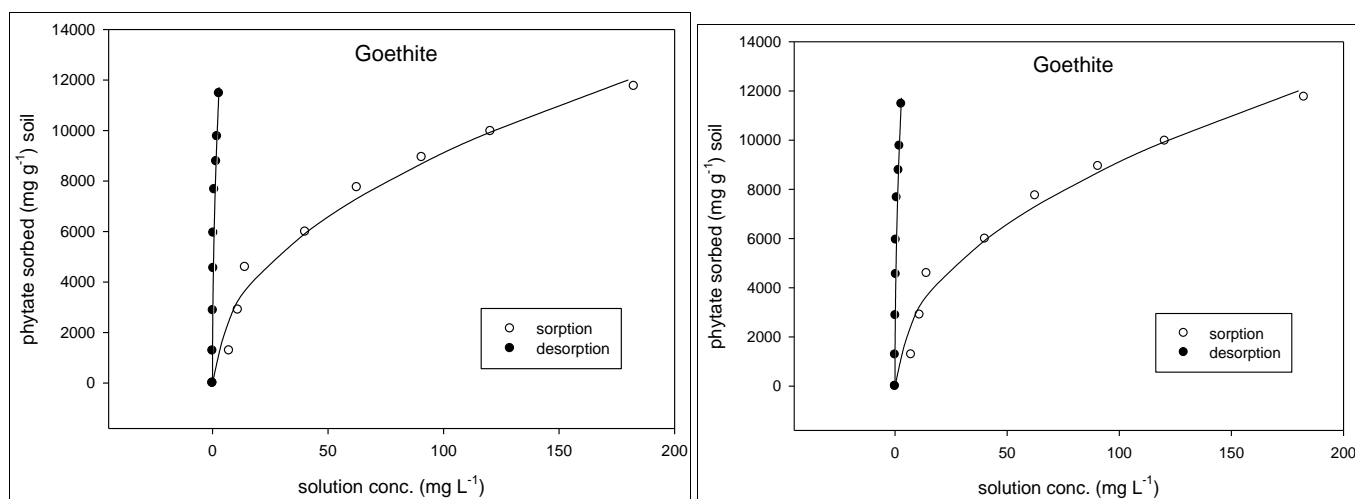


Fig 2: Sorption Desorption curve of goethite and sand

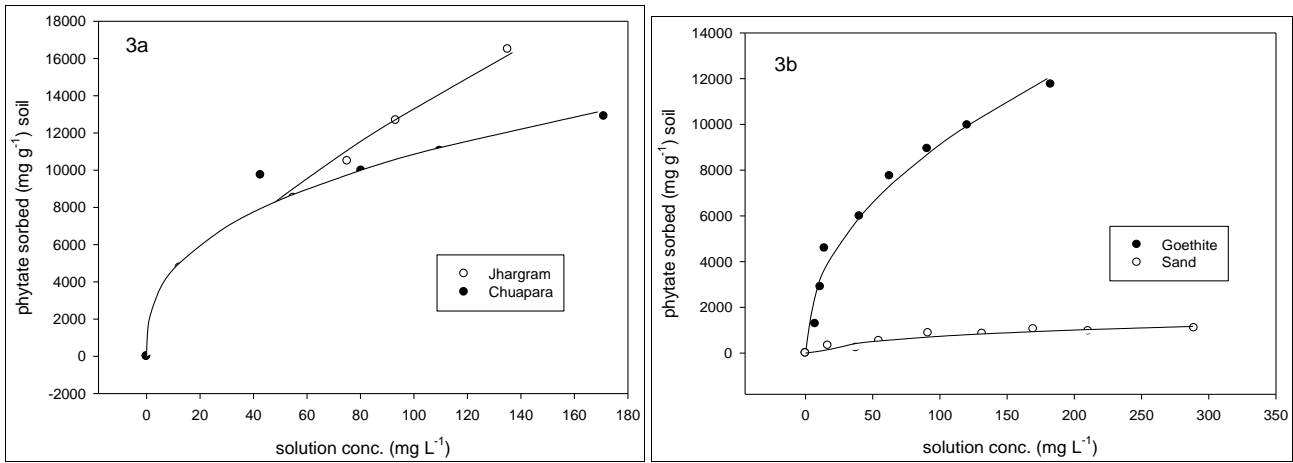


Fig 3: Comparison of sorption curve for between i) soils (3a), ii) goethite and sand (3b)

Table 2: The sorption-desorption parameters of phytate in the studied soils, goethite and sand

	Chuapara	Jhargram	Goethite	Sand
<i>linear term a_s</i>	27646.17	718.166	1047.306	634.119
<i>power term b</i>	0.07264	0.6436	0.4697	0.214
<i>intercept term q_s</i>	29065.01	0.0505	0.0311	964.403
<i>linear term a_d</i>	36606.91	2749.258	7165.143	162.49
<i>intercept term q_d</i>	35732.73	0	0.5055	0.00098
Rs_q	0.9949	0.9981	0.9989	0.9999
<i>hysteresis ratio (a_d/a_s)</i>	δ1.324123	3.828165076	6.841499046	0.2562
A*b for Phytate	2008.218	462.2116376	491.9196282	135.70
<i>instantaneous slope δS/δc at 20mg solution conc.</i>	124.82	158.91	100.45	12.88
Null pt	1.991698	3.52645E-07	2.29756E-10	7.0937

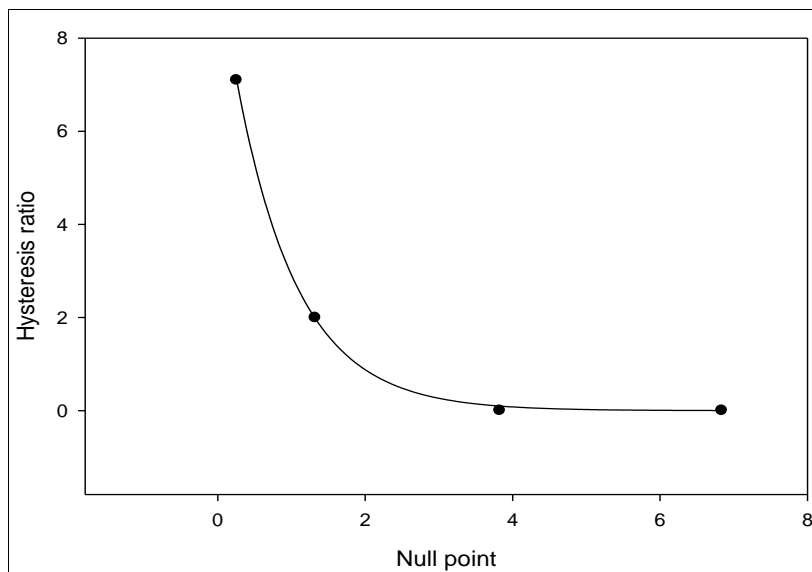


Fig 4: Relationship between the null point concentrations of phytate in solution with hysteresis ratio

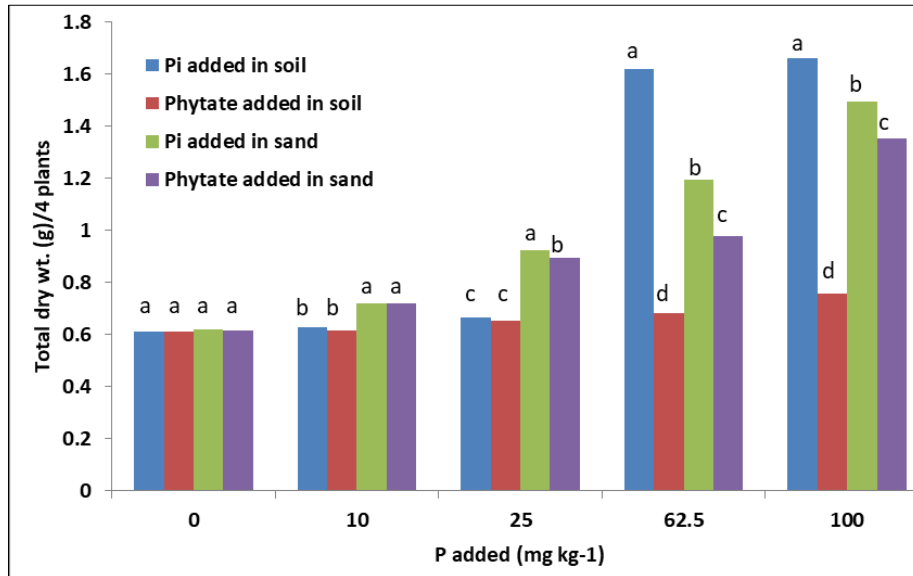


Fig 5: The dry matter weight of Bengal gram under different P treatment in soil and sand.

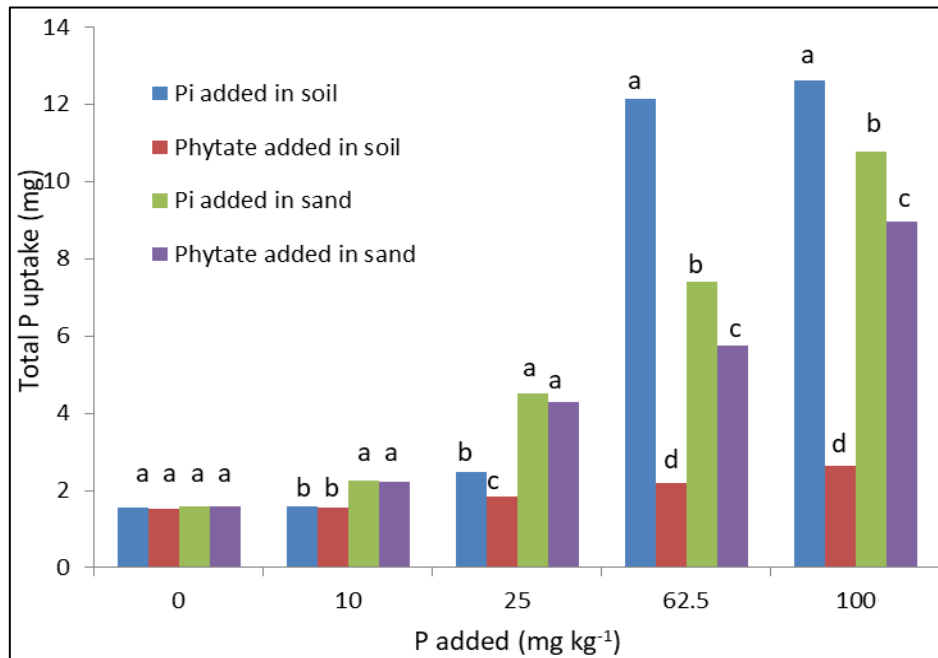


Fig 6: Total uptake of phosphorous under different P treatment in soil and sand

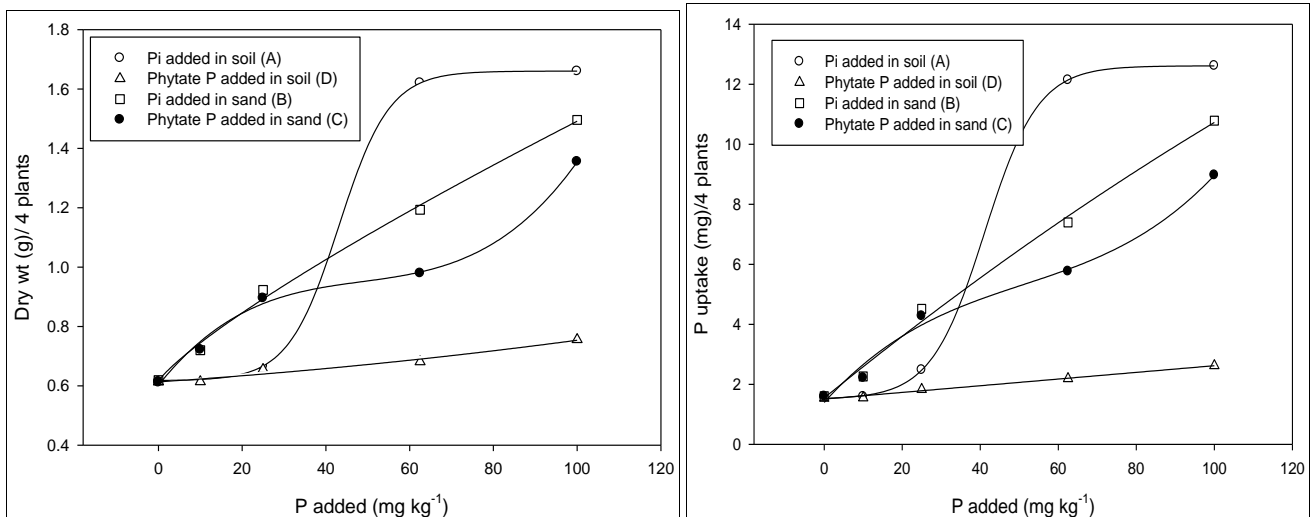
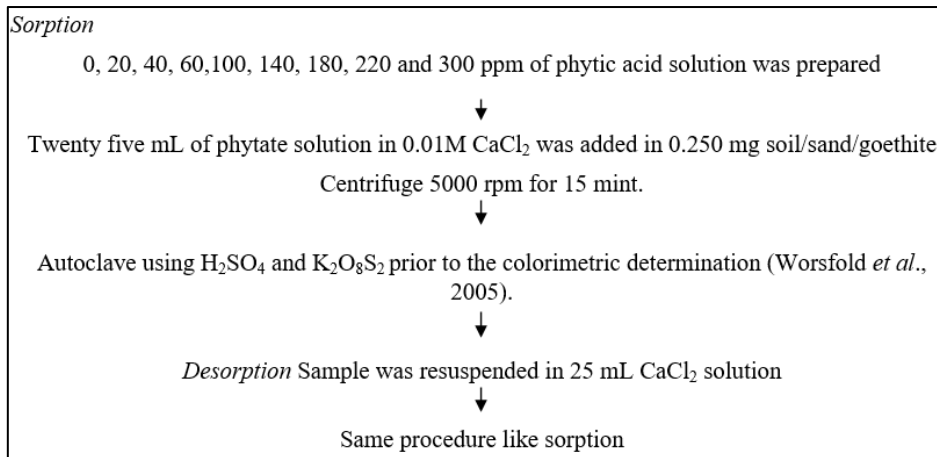


Fig 7: Response curves for dry matter weight and P uptake in Bengal gram against additional P (Pi or phytate)

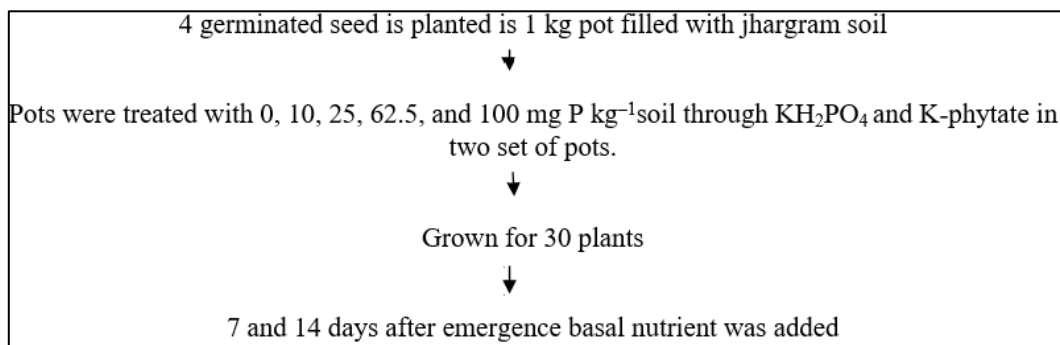
0, 20, 40, 60, 100, 140, 180, 220 and 300 ppm of phytic acid solutions were prepared. Twenty five mL of phytate solution in 0.01M CaCl₂ was added to 0.250 mg soil/sand/goethite samples taken in 100 mml centrifuge tubes and shaken horizontally for 24 h. Two drops of toluene were added. The tubes were centrifuged at 5000 rpm for 15 minutes and the supernatant solution decanted. Phytate in this solution was hydrolysed to inorganic P by digestion in an autoclave using H₂SO₄ and K₂O₈S₂ prior to the colorimetric determination (Worsfold *et al.*, 2005) [17].

For studying phytate desorption, the soil from the solution run (after the supernatant was decanted off) was resuspended in 25 mL CaCl₂ solution and shaken for the same time period and at same temp for the sorption run. Samples were then centrifuged and phytate in the supernatant solution was measured following the same method. Allowance was made, while calculating phytate desorbed, for phytate contained in 3mL of the supernatant carried over from the preceding sorption run and it was mentioned by Sanyal *et al.* (1993) [12].



3mL of the supernatant is taken from sample (Sanyal *et al.*, 1993) Each of the 1 kg capacity earthen pots was filled with soil of Jhargram to which 4 germinated seeds of Bengal gram were sown. The plants in pots were treated with 5 levels of phosphate, viz., 0, 10, 25, 62.5, and 100 mg P kg⁻¹soil through KH₂PO₄ in one set of pots and through K-phytate on the other set of pots. The treatments were replicated twice. Plants were

grown for 30 days with watering regularly to maintain the moisture at 40% saturation level. Seven days and 14 days after the emergence, a nutrient solution was added which gave the following additions as basal nutrient (mg nutrient kg⁻¹): manganese-4 mg, molybdenum-1 mg, copper-1 mg, boron-0.2 mg, zinc-10 mg, potassium-100 mg, magnessium-6 mg, nitrogen-100 mg.

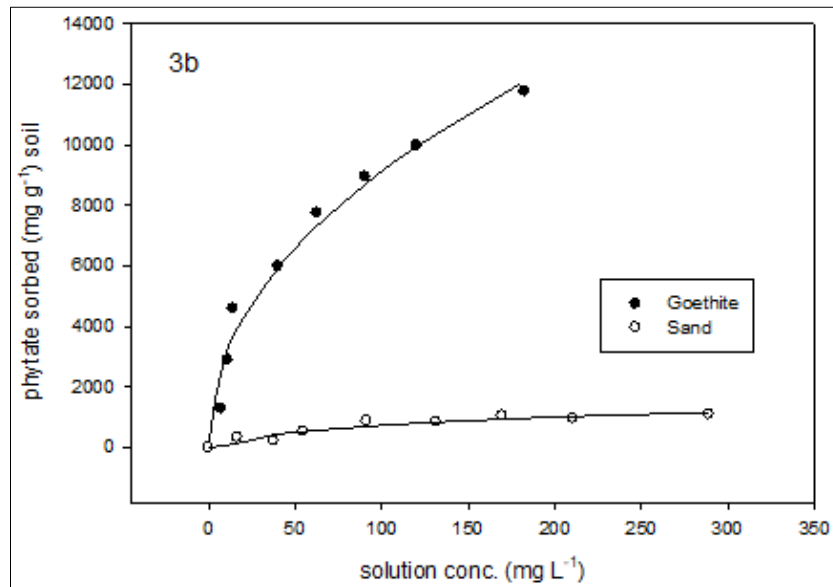


The phosphorus in plant sample was determined by a wet oxidation method using tri acid digestion: HNO₃:H₂SO₄:HClO₄ in the ratio of 10:1: 4 as described by Singh *et al.* (2005) and measured using the vanadomolybdate method.

The Freundlich equation, modified by adding an intercept term (q) (Barrow, 2008) was fitted to the data:

$$S=ac^b-q \tag{1}$$

Where S = sorption of phytate in mg kg⁻¹, C= the observed solution concentration in mg phytate L⁻¹, and a, b, q are parameters. The intercept (q) is formally the value for desorption at zero solution concentration of phytate.



The aim of this study was to compare sorption of phytate in pure and applied system *viz.* goethite and sand on one hand and soils on the other hand. Phytate is a six phosphate monoester available in larger quantity than other organic forms of P in soil. Therefore, it was also to study as to what extent the sorbed phytate could be utilized by crop when compared with an inorganic source of P since P of phytate is hydrolysed by the action of enzymes having originated from plants roots and microbes in soil.

The soil of Chuapara Tea Garden had a high value of Colwell P. This is because of the history that emphasizes that despite soil acidity, this soil was regularly applied with both soluble and sparingly soluble forms of P for over 100 years being the average age of the tea garden in this area. On the contrary the Jhargram soil was collected from uncultivated land having no history of P fertilization as ever. It was a P deficient soil which may be to some extent comparable with pure goethite in terms of high value of adsorption characteristics. Because of the high value of Colwell P in the Chuapara soil, the extent of adsorption of phosphate had an adverse effect on the sorption of phytate which has become evident from the lowest value of the power term "b". While comparing the instantaneous slope of the curves at 20 mg solution concentration L⁻¹, which is an indication of the sorptive ability, it was noted that the Jhargram soil had highest affinity for sorption of phytate than others.

The value of the hysteresis ratio is a measure of the ability of surface to retain the sorbed phytate in it. Inositol hexaphosphate or phytate act as strong ligands owing to their high anionic charge. It is strongly adsorbed on to the surfaces of ferric oxides (De Goot and Golterman, 1993) [8]. These authors have noted that addition of phytate to a suspension of orthophosphate adsorbed on to FeO(OH) released orthophosphate. Afterwards, the chemical reduction of FeO(OH)- phytate leads to formation of insoluble Fe₄(phytate) rather than releasing free phytate; suggesting an irreversible binding of phytate under this condition and hence high hysteresis. In soil, displacement of orthophosphate by phytate has been observed (Anderson and Malcolm, 1974) [2]; the likely mechanism of which was competition for the same binding site. This made some of the binding sites accessible to phytate in contrast to all free sites available in goethite. Phytate is supposed to occupy an area equivalent to four sites for Pi. In other words, it is bound to the oxide by four of its

six phosphate groups. The other two phosphate groups would be free and would increase the negative charge of the surface (Celi *et al.*, 1999) [7]. According to these authors, adsorption of organic P compounds is determined by the number of P groups occupying surface binding sites. Thus, orthophosphate diesters are weakly adsorbed than monoester phytate rendering the former vulnerable to action of enzyme hydrolysis. In the present study, low hysteresis ratio for phytate in tea garden soil could be explained by the high phosphate status of the soil competing with phytate for binding sites. Sand having largely devoid of sorption sites could not retain phytate during desorption and hence low hysteresis.

In the pot experiment, the dry matter weight and uptake of P was recorded using two sources of P and two media of growth under five different applications of P. The dry matter weight of the plants and P uptake both exhibited similar pattern of response curves against applied P because the soil of Jhargram is acidic and high in sorption capacity, irrespective of P sources. The dry matter weight and P uptake were significantly less for want of available P than in sand medium. The latter making the added P soluble in absence of binding sites. Phosphorous of phytate is enzymatically hydrolysed. The enzymes responsible are both plant and microbial origin. In absence of microbes in sand culture, enzymes released from plant roots might have played role in hydrolyzing phosphates to make it plant available under P stress (Terafdar and Classen, 1988) [14]. This mechanism was responsible upto 25 mg P kg⁻¹ to lead to higher values of the parameters in sand irrespective of sources. At rates of P application higher than this the plants were subjected to excess of soluble P whether in soil or sand. However advantage of soil to be medium of growth of plant over sand led to generate highest dry matter weight and P uptake in Bengal gram.

The two major determinants of the availability of organic P to plants are generally suggested to be (a) solubility of the particular source and (b) its susceptibility to plants and soil phosphatases (Adam and plate, 1992) [1]. Addition of organic and inorganic P to a strongly fixing red earth soil did not increase P availability to plants. Because of very low organic matter in the Jhargram soil, enzyme phosphatase of plant root origin played active role in P nutrition of crop in phytate treated soil.

Conclusion

From the present study, it was confirmed that Chuapara soil from Tea Garden of North Bengal had high water holding capacity and organic carbon together with high value of Colwell P, whereas soil from Jhargram was from uncultivated land. In soils and in goethite desorption curves were not close to sorption curves, showing appreciable hysteresis and high buffering capacity while sand showing little hysteresis and poor buffering capacity for phytate for want of specific binding sites. The tea garden soil showed low hysteresis ratio for phytate which leads to the high phosphate status of the soil. Addition of organic and inorganic P to a strongly fixing red earth soil did not increase P availability to plants. Because of very low organic matter in the Jhargram soil, enzyme phosphatase of plant root origin played active role in P nutrition of crop in phytate treated soil. The finding from the present study suggested that organic P in the form phytate is a good source of P and sometimes better than the inorganic soluble forms. Phytate is strongly adsorbed on to the binding sites of soil particles rendering it unavailable in soil solution and thereby hydrolysis by the enzymes of microbial or plant root origin. Further research should be aimed at finding ways and means to hydrolyse P of phytate that is adsorbed on to soil components.

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