Annals of Plant and Soil Research 23 (4):390-396 (2021)
https://doi.org/10.47815/apsr.2021.10089

Evaluation of phosphorus availability indices in acid Alfisols

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Received: May, 2021; Revised accepted: June, 2021

ABSTRACT

To identify the most appropriate method of available phosphorus extraction from acid Alfisols of north east India, greenhouse pot experiments were conducted with two acid Alfisols (non-limed and limed), amended with six doses of P fertilization. Maize (Zea mays), as the test crop, was grown for 45 days, and the plant response was regressed against soil P availability estimated using 07 chemical extractants viz. Bray-1, Bray-2, Mehlich-1, Mehlich-3, Olsen's reagent, water, and $CaCl_2$. Suitability of extractants was adjudged based on their predictive ability (R^2 values). Bray-1, Bray-2 and Mehlich-3 were the best predictors of plant dry matter yield and P uptake in both the soils, with their predictive ability being very close to each other. Mehlich-3 P was strongly correlated with Bray-1 values ($r = 0.90^{**}$ and 0.99^{**} in untreated and limed soil, respectively). Limed soil had 33, 37 and 47% less Bray-1, Bray-2 and Mehlich-3 P, compared to non-limed soil. Averaged across the three best extractants (i.e. Bray-1, Bray-2, and Mehlich-3), critical P availability in limed soil was 32% lower than in untreated soil. Critical limits of plant P concentration ranged from 0.20 to 0.24%. Similar effectiveness in available P extraction and strong correlations between Bray-1 and Mehlich-3 values suggest that Mehlich-3 can effectively replace Bray-1 extractant without affecting the reliability of P testing in acid soils. Being a multi-nutrient extractant, adoption of Mehlich-3 can improve the rapidity and economy of soil testing.

Keywords: Bray-1, Critical limits, Mehlich-3, Soil testing, Universal extractant

INTRODUCTION

Acid soils occupy nearly one-third of cultivated land in India. Soil acidity is particularly severe in Northeastern India where more than 95% of the soils are acidic in reaction (Kumar *et al.,* 2021a). Amidst the multitude of acidity-related fertility constraints, phosphorus (P) deficiency is of vital concern (Kumar *et al.,* 2012; Singh *et al.,* 2014; Marwein *et al.,* 2017). Adequate P fertilization is therefore indispensable for improving crop productivity in acid-soil regions of India (Bhattacharjee *et al.,* 2013; Kumar, 2015). Reliable estimate of plant-available P and precise knowledge of its critical limit in soil are essential for determining the rate of P fertilization in order to optimize crop yield and quality, and to minimize the adverse impacts of excess P on environment and farm economy.

Soil P testing involves extraction of available P by a suitable extractant, P estimation in extract, and interpretation of the soil test result. Knowledge of the critical P requirement of a crop is also essential for precise recommendation of P fertilization. Clearly, the first requirement is selection of suitable P extractant. A large number of chemical extractants (for example, Bray's reagents, Olsen's reagent, water, 0.01*M* $CaCl_2$, Mehlich reagents, Morgan reagent *etc*.) are used in different countries and regions based on their suitability to the local soils, and crop P uptake correlations. Since multiple elemental analysis is possible these days using sophisticated instruments (such as ICP and AAS), focus of late has shifted on adoption of extractants capable of multiple-element extraction, commonly known as universal extractants. Water, 0.01*M* $CaCl_2$, Mehlich 1 and 3 are some of the commonly known universal extractants used for multiple element extraction, including phosphorus.

In India, Bray-1 reagent (0.025*N* HCl + 0.03*N* NH_4F) is used for available P extraction from acidic soils. The reagent however lacks multi-nutrient extraction capability. Also, it has not been evaluated sufficiently against other alternative extractants available, which necessitate its evaluation *vis-à-vis* other alternative P extractants for its suitability in soil P testing. It is also important to explore if any multi-nutrient extractant, including water, 0.1 M $CaCl_2$ and Mehlich-3, could perform better or at least as good as Bray-1. Critical P availability in soil and P concentration in plant has also not been

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adequately studied in the acidic soils of India, which merits an investigation. Thus, in view of the limitations of the present way of soil P testing and the research gaps identified, the present study was conducted to evaluate the soil test methods for available phosphorus and to determine the critical limits of P availability in acid Alfisols of northeastern India.

MATERIALS AND METHODS

Greenhouse pot experiments, with maize (*Zea mays* L.) as the test crop, were conducted with two Alfisols (limed and not-limed), collected from the experimental farm of the Soil Science Division, ICAR Research Complex for NEH Region, Umiam, Meghalaya. The plot from which the limed soil was collected received lime @ 2.5 t ha^{-1} one year ahead of bulk soil collection.

Table 1: Characteristics of the experimental soils

Parameters	*Alfisol-1	*Alfisol-2
pH (1:2 soil/water)	4.49	5.80
Organic carbon (%)	2.01	2.03
Texture	Sandy loam	Sandy loam
Bray1 P (mg/kg)	18.8	12.0
Total acidity (meq/100g)	4.45	2.30
Exch. Acidity (meq/100g)	2.70	0.12
Exch. Al (meq/100g)	2.37	0.11
Citrate dithionite extractable Fe (%)	1.23	0.6
Exchangeable Ca (meq/100g)	0.4	3.4
Exchangeable Mg (meq/100g)	0.2	1.2
Effective CEC (meq/100g)*	3.9	6.4
Specific surface area (m^2/g)*	22.2	24.5
Specific charge density (mol/m^2) ($x10^{-6}$)	1.75	2.61

**Soils were collected from two different plots in the same field; soil 1 was never limed while soil 2 was limed @ 2.5t $CaCO_3$ ha^{-1}one year before collection for the pot experiments*

The bulk soils were dried and passed through 5 mm sieve before using them for pot experiments. Maize (variety DA 61A) as the test crop was grown in 4.0 kg capacity pots filled with 3.0 kg of each experimental soils (two Alfisols and one Inceptisol) amended with six doses of P fertilization @ 0, 10, 20, 40, 80 and 160 mg P kg^{-1} soil. Six doses of P fertilization were used to create P fertility gradient in experimental soils for observing plant response to available P in a sufficiently wide range of P availability. Analytical grade KH_2PO_4 was used as P source which was applied to the soils in solution form. Uniform doses of N (@ 36 mg kg^{-1}) as urea, and K (@ 18 mg kg^{-1}) as KCl was applied in all the pots in solution form. The doses were relatively low as the plants were to be grown only up to 45 days. The initial physico-chemical properties are presented in Table 1.

After filling the pots with experimental soils, five seeds of maize were sown per pot, which was thinned down to three after germination. Pots were watered regularly to avoid moisture stress. Pots were also rotated regularly for their true randomized placements. Plant tops were harvested 45 days after sowing, oven-dried at 70°C for till constant weight and dry weights were recorded. Plant P concentration was determined colorimetrically after wet digestion, and P uptake was estimated by multiplying plant biomass with P concentration. Soils were taken out of the pots and carefully separated from the roots. Soil samples were then air-dried and passed through 2.0 mm sieve for P extraction. Seven chemical extractants *viz*. Bray-1, Bray-2, Mehlich-1, Mehlich-3, Olsen's reagent, water, and $CaCl_2$ were used for soil P extraction (Table 2). Available P thus extracted was correlated with dry matter yield, plant P concentration and total P uptake. Suitability of extractants was adjudged based on their strength of correlations with plant response and P uptake. Critical limit of available P in soil and P concentration in plant was determined using graphical method of Cate and Nelson (1971). Scatter diagram, taking relative yield on Y-axis versus available P on X-axis was plotted to assess the critical level of available P below which probability of obtaining adequate response to added P fertilizer remains high. In estimation of the critical limits for all the extractants, instead of taking a fixed value of relative yield at 80 or 90%, horizontal and vertical lines were drawn in such a way that most values fall either in 1^{st} or 3^{rd} quadrants. The data obtained were statistically analyzed using SPSS version 16.0 software. Duncan Multiple Range Test was used for comparing treatment means. Significance of treatments' effect was tested at 5% level of probability.

Table 2: Composition and procedural details of the chemical extractants for available P

Extractants/ Reagents	Chemical Composition	Soil/solution Ratio	Shaking time & frequency	References
Bray 1	0.025*N* HCl + 0.03N NH_4F	1:10	5 min, 140rpm	Bray and Kurtz (1945)
Bray 2	0.1*N* HCl + 0.03N NH_4F	1:10	5 min, 140rpm	Bray and Kurtz (1945)
Olsen	0.5*N* $NaHCO_3$	1:20	30 min, 140rpm	Olsen *et al.* (1954)
Mehlich 1	0.05*N* HCl + 0.025*N* H_2SO_4	1:10	5 min, 180rpm	Mehlich (1953)
Mehlich 3	0.2*N* CH_3COOH + 0.25*N* NH_4NO_3 +0.015*N* NH_4F + 0.013*N* HNO_3 + 0.001*M* EDTA	1:10	5 min, 180rpm	Mehlich (1984)
Water	Deionized water	1:10	2hrs, 140rpm	Kuo (1996)
$CaCl_2$	0.01 *M* $CaCl_2$	1:10	2hrs, 140 rpm	Kuo (1996)

RESULTS AND DISCUSSION

Irrespective of the chemical extractants used, P availability indices increased with increasing level of P application, though the trends were not as pronounced with $CaCl_2$ and water extraction. In both the soils, Bray-2 extracted highest amount of P followed by Bray-1, whereas $CaCl_2$ and water extracted the least. Mehlich-1, Olsen's reagent and Mehlich-3 were moderate in P extractability (Table 3). Averaged across the soils, Bray-2 extracted 19% more P while Mehlich-3 yielded 32% less P as compared to Bray-1. In general, limed Alfisol (Alfisol-2) had lesser quantity of available P than the non-limed Alfisol. Averaged across the P doses, limed Alfisol had 33, 37 and 47% less Bray-1, Bray-2 and Mehlich 3 P, respectively compared to non-limed Alfisol. The observed variation in P extractability of different extractants can be explained by the strength of chemical reagents used therein. Bray-2 extracted highest amount of soil P as the strong acid extractant (0.1*N* HCl + 0.03*N* NH_4F) can dissolve Al and Fe bonded P, releasing thereby available P into the soil solution. The higher solubility in Bray-2 P may be due to its higher strength of acidity and complexion of Al^{3+} and Fe^{2+} ions with F^- ions and consequent release of P adsorbed by these trivalent ions. Strength of acidity in Bray-1 (0.025N HCl) is much lesser than in Bray-2 (0.1*N* HCl) which explains its relatively weaker extractability. In many previous studies, Mehlich-3 was reported to extract more P than Bray-1 in some soils (Gutierrez Boem *et al.* 2011), similar in other soils (Kleinman *et al.* 2001), and lesser P in others (Herlihy *et al.,* 2006). We however observed consistently lesser values of Mehlich-3 P than the Bray-1, as reported by Herlihy *et al.* (2006) in temperate grassland soils, unlike subtropical agricultural soils in our study.

Table 3: Available phosphorus (mg kg^{-1}) extracted by seven chemical extractants at various levels of P fertilization

	P levels (mg kg^{-1})	Bray-1 P	Bray-2 P	Olsen-P	Mehlich-1 P	Mehlich-3 P	Water-P	$CaCl_2$-P
Alfisol-1	0	29.4^{f}	42.6^{c}	17.1^{c}	28.2^{d}	33.4^{c}	1.42^{b}	1.46^{b}
	10	33.4^{e}	46.4^{c}	26.8^{bc}	36.0^{cd}	37.1^{bc}	1.59^{b}	1.10^{bc}
	20	41.4^{d}	50.4^{c}	25.4^{bc}	40.0^{bc}	39.3^{bc}	1.46^{b}	1.14^{bc}
	40	50.0^{c}	59.2^{bc}	34.4^{b}	45.5^{b}	44.5^{b}	1.53^{b}	0.56^{c}
	80	58.5^{b}	70.1^{b}	29.7^{bc}	44.9^{b}	45.6^{b}	4.21^{a}	2.98^{a}
	160	85.7^{a}	95.5^{a}	50.9^{a}	64.5^{a}	62.0^{a}	4.34^{a}	3.50^{a}
	Mean	*49.7^{b}*	*60.7^{a}*	*30.7^{d}*	*43.2^{c}*	*43.7^{c}*	*2.4^{e}*	*1.8^{f}*
Alfisol-2	0	14.0^{e}	22.9^{e}	8.3^{e}	16.2^{e}	12.1^{e}	1.51^{b}	0.40^{a}
	10	17.8^{e}	27.1^{d}	19.2^{d}	18.5^{de}	13.7^{de}	1.72^{ab}	0.37^{a}
	20	22.4^{d}	28.4^{d}	19.3^{d}	20.4^{d}	15.0^{d}	1.46^{b}	0.36^{a}
	40	28.4^{c}	36.7^{c}	26.4^{c}	24.5^{c}	19.6^{c}	1.77^{ab}	0.23^{a}
	80	42.7^{b}	43.6^{b}	35.8^{b}	39.3^{b}	29.2^{b}	2.37^{ab}	0.27^{a}
	160	75.9^{a}	70.4^{a}	46.7^{a}	59.6^{a}	48.8^{a}	2.74^{a}	0.37^{a}
	Mean	*33.5^{b}*	*38.2^{a}*	*26.0^{d}*	*29.8^{c}*	*23.1^{e}*	*1.93^{f}*	*0.33^{g}*

Values within a column in each soil, and the mean values (in lower most raw in each soil) followed by common letter/s are not significantly different (P<0.05) by Duncan's Multiple Range Test

As per general perception, lime application is recommended to increase P availability in acid soils by inactivation of Al and Fe in soil solution; we however observed significant decline in all P availability indices in limed Alfisol. It is reported that pH is the most dominating factor in determining P extractability (Haque *et al.,* 2013), which is hugely altered by soil liming. Lime-induced decline in P availability may be attributed to the adsorption or precipitation of applied P as calcium phosphate. In a latest study, Kumar *et al.* (2021b) has also confirmed the lime induced decline in soil P availability in acidic soil of northeastern India.

Table 4: Plant response to phosphorus fertilizations in experimental soils

P levels ($mg\ kg^{-1}$)	Alfisol-1			Alfisol-2		
	Dry matter yield ($g\ pot^{-1}$)	P Conc. (%)	P uptake ($mg\ pot^{-1}$)	Dry matter yield ($g\ pot^{-1}$)	P Conc. (%)	P uptake ($mg\ pot^{-1}$)
0	11.8^{c}	0.16^{d}	19.8^{d}	11.1^{e}	0.20^{c}	21.6^{d}
10	13.2^{bc}	0.17^{cd}	22.4^{d}	12.5^{de}	0.22^{bc}	27.5^{cd}
20	13.6^{bc}	0.19^{bc}	25.^{cd}	13.3^{cd}	0.23^{ab}	30.9^{c}
40	15.8^{bc}	0.22^{a}	33.7^{bc}	14.9^{c}	0.23^{ab}	33.9^{c}
80	17.1^{b}	0.21^{ab}	35.7^{ab}	18.2^{b}	0.23^{ab}	42.4^{b}
160	21.2^{a}	0.20^{ab}	43.8^{a}	22.4^{a}	0.25^{a}	56.2^{a}
Mean	*15.5*	*0.19*	*30.1*	*15.4*	*0.23*	*35.4*

Values within a column followed by common letter/s are not significantly different (P<0.05) by Duncan's Multiple Range Test

Significant increases in dry matter yield and P uptake were recorded with increasing P doses in both the soils, particularly at higher levels of P supply (Table 4). In limed Alfisol, plant P concentration and P uptake were relatively higher than in untreated Alfisol, though there was no wide difference in dry matter yield for both soils. The increased P uptake and dry matter yield in lime-treated Alfisol may be due to lime-induced increases in soil pH, reduction of Al^{3+} toxicity and thereby improved root growth. It is widely reported that lime application increases the phyto-availability of essential nutrients and ameliorates the other acidity-induced fertility constraints on acid Alfisol (Kumar *et al.,* 2012). Kumar *et al.* (2021b) has also reported improved plant growth and yield in limed soil despite lower levels of P availability caused by liming. Considering the better growth and crop yield despite the reduced P availability caused by liming, Kumar *et al.* (2021b) reasoned that the liming improved plant growth not by increasing soil P availability but by improving the plant's ability to acquire P and other nutrients more efficiently from a lime-treated soil; better growth and proliferation of root system due to reduced Al toxicity must understandably be the reason behind.

Table 5: Coefficient of determination (R^2) for the relationship between plant responses and phosphorus availability indices in experimental soils

		Bray 1	Bray 2	Olsen	Mehlich 1	Mehlich 3	Water	$CaCl_2$
Alfisol-1	Dry matter yield	0.73**	0.78**	0.58**	0.68**	0.78**	0.60**	0.61**
	P uptake	0.79**	0.82**	0.59**	0.66**	0.80**	0.54**	0.57**
Alfisol-2	Dry matter yield	0.93**	0.95**	0.87**	0.93**	0.94**	0.47**	ns
	P uptake	0.91**	0.93**	0.87**	0.90**	0.91**	0.41*	ns

**P < 0.05; **P < 0.01; ns - not significant*

Predictive ability of P extractants in terms of R^2 values are is presented in Table 5. Bray-1, Bray-2 and Mehlich-3 were the best predictors of plant dry matter yield, and P uptake in both the soils, with their predictive ability being very close to each other. Coefficients of determination for Water and $CaCl_2$ were non-significant in limed Alfisol and poor in untreated Alfisol. Considering the similar effectiveness of these three extractants, Mehlich-3 could be advocated as a better alternative than the other two, given its multi-nutrient extraction capability. Being a universal extractant, Mehlich-3 can have some definite advantages over Bray-1 and Bray-2, as the former can extract many other secondary and micronutrients simultaneously in addition to

P, unlike Bray-1 and Bray-2 which are used for extraction of P only (Mehlich, 1984). If adopted in routine soil testing, Mehlich-3 can considerably reduce the time, cost, and labor requirements in soil testing, making them an obvious choice over single-element extractant. However, its multi-nutrient extraction efficiency also needs to be examined in all acidic soil types of India. Confirmation of its P extraction efficiency and similar predictive ability as Bray-1 and Bray-2 in strongly acidic soils (pH 4.49) in our experiment lends further credibility to Mehlich-3 as a better alternative P extractant in routine soil testing. As Mehlich-3 P was strongly correlated with Bray-1 values (r = 0.90** and 0.99** in untreated and limed Alfisol, respectively (Table 6), the currently used Bray-1 reagent can be safely replaced with Mehlich-3 without compromising with the reliability of P estimation in routine soil testing.

Table 6: Correlations (R-values) of phosphorus availability indices in experimental soils

		Bray 1	Bray 2	Olsen	Mehlich 1	Mehlich 3	Water
Alfisol-1	Bray 2	0.878**					
	Olsen	0.789**	0.792**				
	Mehlich 1	0.915**	0.880**	0.784**			
	Mehlich 3	0.908**	0.831**	0.816**	0.776**		
	Water	0.794**	0.802**	ns	0.692**	0.669**	
	$CaCl_2$	0.749**	0.736**	0.516*	0.586*	0.693**	0.851**
Alfisol-2	Bray 2	0.985**					
	Olsen	0.915**	0.927**				
	Mehlich 1	0.992**	0.979**	0.929**			
	Mehlich 3	0.996**	0.988**	0.922**	0.995**		
	Water	0.694**	0.613**	0.534*	0.685**	0.662**	
	$CaCl_2$	ns	ns	ns	ns	ns	ns

**P < 0.05; **P < 0.01; ns - not significant*

Critical limits of phosphorus in soil varied with the method of phosphorus extraction. According to graphical method of Cate and Nelson (1971), the critical limits of Bray-1, Bray-2 and Mehlich-3 P in untreated Alfisol were 59, 75 and 52 mg P kg^{-1} soil, respectively compared to 48, 47 and 31 mg P kg^{-1} soil in limed Alfisol (Fig. 1).

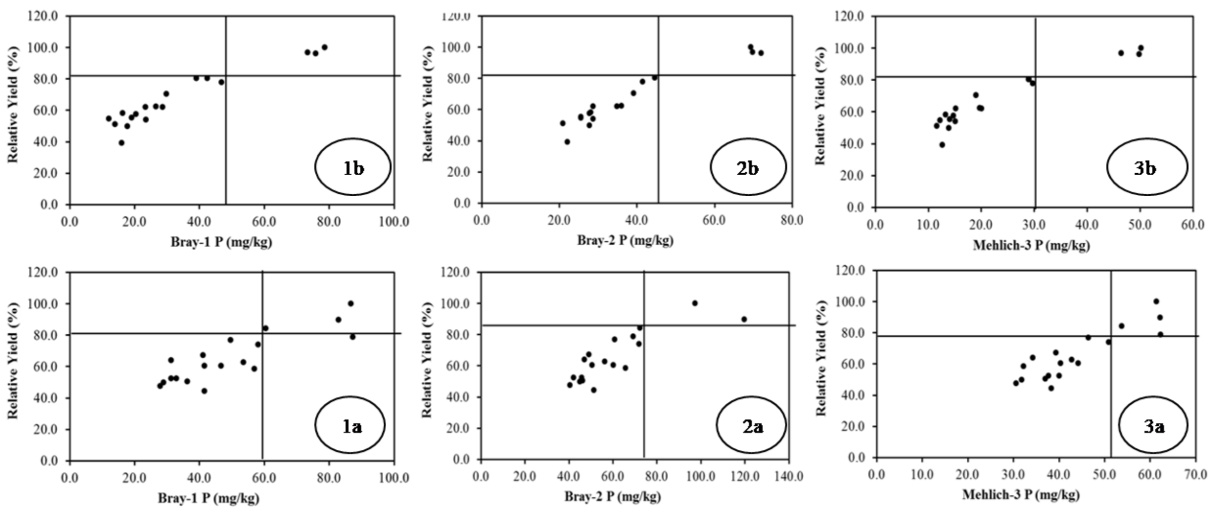

Fig. 1: Critical limits of Bray-1, Bray-2 and Mehlich-3 phosphorus in (a) Alfisol-1 (b) Alfisol-2

Averaged across the three extractants, critical P availability in limed soil was 32% lower than in untreated soil. The results imply that liming improves plant growth not by increasing soil P availability but by reducing the critical requirement of P availability in soil, suggesting the improved plant's ability to acquire P and other nutrients from a lime-treated soil. This can be attributed to better growth and proliferation of root system due to reduced Al toxicity in lime treated soil (Kumar *et al.,* 2021b). Since, Bray-1, Bray-2 and Mehlich-3 were the three best extractants, critical values for other extractants are not discussed here. Below these limits, economic response to P fertilization could be expected as they discriminate P responsive soils from non-responsive ones. Utility of critical limits of P availability in soil, and the similar inferences on discriminating P responsive soils from non-responsive ones are well established in literature (Bado *et al.,* 2010; LaBarge, 2013; Meetie *et al.* 2015), which needs no further elaboration. Like in soil, critical limit of P concentration in plant (maize) was also calculated (45 days after showing), and was found to be 0.20% and 0.24% in untreated and limed Alfisol (Fig. 2). Below this concentration, plant could be regarded P deficient at the specified period of plant growth, suggesting a need of additional P fertilization. Nearly same range of plant P concentration (0.22 %) was previously reported to be critical (Meetie *et al.,* 2015) in soybean grown in acid soil of northeastern region.

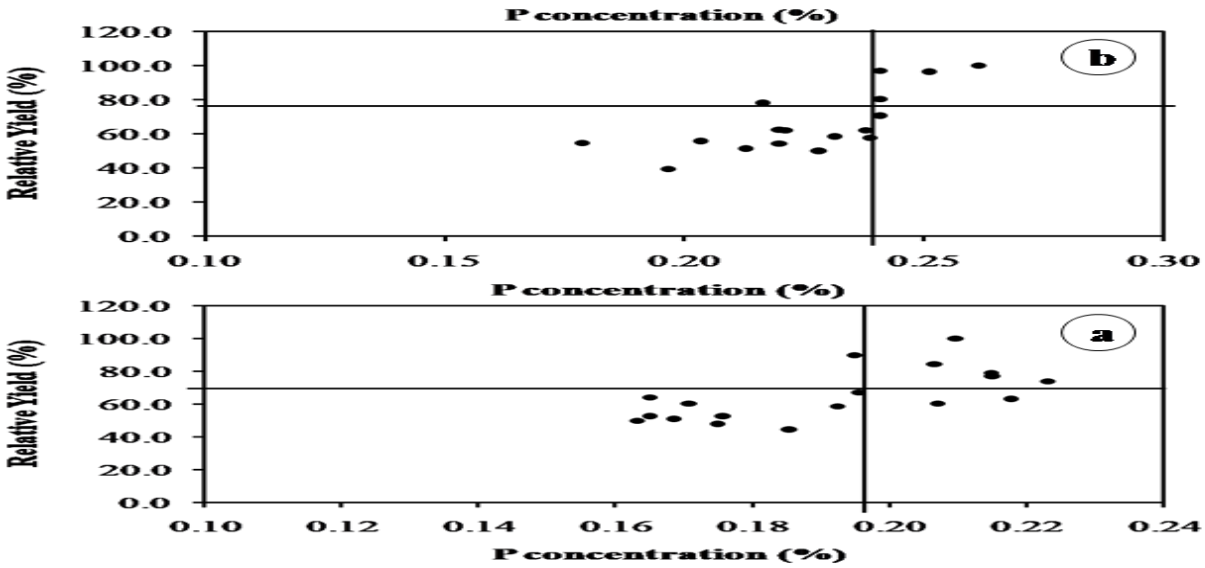

Fig. 2: Critical P concentration in maize grown on: (a) Alfisol-1 (b) Alfisol-2

The critical P availability in soil as established by our study is much higher than the currently used values for acidic soils of northeastern India, which suggests that the currently applied values of critical P availability in soil might be giving misleading results regarding deficiency and sufficiency of available P. Based on similar assumptions, Kumar (2015) examined the phosphorus requirement of acidic soils in northeast India using phosphate sorption isotherms, and concluded that actual P requirement was much higher than the currently recommended values. Results of our study, supported by the previous findings of Kumar (2015), clearly underline the need for revising P fertility classes and P recommendations for crop production in acidic soils of northeast India.

To sum up, Mehlich-3 extractant had similar ability to predict soil P availability as Bray-1, and Mehlich-3 P also correlated strongly with Bray-1 P values. Thus Mehlich-3 can replace Bray-1 extractant without affecting the reliability of P testing in acidic soils. Being a multi-nutrient extractant, adoption of Mehlich-3 can improve the rapidity of soil testing, saving substantial amount of time, cost and labor involved therein. Potential utility of Mehlich-3 as multi-nutrient extractant for acidic soils of India needs to be tested by further experimentations. Relatively higher value of critical P availability found in our study suggests that P fertility classes and P recommendations for crop production needs to be re-established in acidic soils of northeast India.

ACKNOWLEDGEMENT

Authors are thankful to the ICAR-RC for NEH Region, and CPGS, CAU, Umiam for supporting this work.

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