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Thirteen Year Long Term Fertilization Effect on Soil Phosphorus Fractions of an Acid *Inceptisol* and Their Contribution to Phosphorus Uptake by a Double Crop of Rice under Sub-Tropical Climate

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A 13 year old long term fertilizer experiment conducted on an acid soil with rice-rice system was used to study the impact of continuous application of fertilizer nutrients and amendments on changes in soil phosphorus fractions and their relative contribution to P nutrition. There was accumulation of total P on surface soil in all P fertilized treatments and depletion in P minus treatments. Depending on the P balance the treatments differed significantly with respect to P accumulation. 150%NPK treatment had highest P accumulation (841.92kgha^{-1}) in surface soil. The high yielding treatments *viz.* 100%NPK+FYM and 100% NPK +FYM+Lime had relatively less P built

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up than 100%NPK. The total P comprised of inorganic P, 64.43-89.60% and organic P, 10.40–35.57%. The inorganic fraction in terms of their abundance were in the order: Sal-P (42.13 kgha^{-1}) <Occl-P (52.16 kgha^{-1}) < Al-P (52.98 kgha^{-1}) < Ca-P (55.44 kgha^{-1}) < Red–P (134.22 kgha^{-1}) < Fe-P (218.70 kgha^{-1}). Olsen P which was significantly influenced by the fertilizer treatments had a very strong positive correlation ($p<0.01$) with Sal-P(r= 0.469***), Occl-P(r=0.639***) and Ca-P (r=0.739***). Among the inorganic P fractions, Ca-P contributed highest of 42.57% to P uptake, followed by Occl-P (28.59%), Sal-P (18.56%) and Al-P (7.63%). The fractions in order of their relative importance for P uptake under submerged rice-rice situation are Ca-P > Occl-P > Sal-P > Al-P > Red-P > Fe-P. The study indicated that 100%NPK+FYM and 100% NPK+FYM+Lime are the best treatments for maintaining more active fractions of P required for P nutrition of rice-rice system under the tropical acid situation.

Keywords: Rice-rice system; phosphorus fractions; phosphorus uptake; long term fertilization; FYM.

1. INTRODUCTION

Rice–rice cropping system is most prevalent across a major portion of India as well as South Asia, especially among small and marginal farmers. It is usually practiced by farmers where either sufficient irrigation is available or in favorable lowland rain fed areas [1,2]. In addition to irrigation water availability, high consumer demand, a relatively stable market price, and assurance of a minimum support price by the government encourage the farmers to grow two crops of rice continuously in consecutive seasons.

In many of these rice production areas, particularly in highly weathered soils of low latitude regions phosphorus (P) has become a limiting nutrient after nitrogen (N) [3]. Although the total P content of soils may be large, only a small part of it is available for plant uptake [4,5]. Most arable soils cannot supply sufficient amount of the element to the crops. Therefore P fertilizer is applied every season to overcome the deficiency. When fertilizer is applied to soil, only a small amount (10-30%) is taken up by the crop and a major part remains in soil which is not available to the present crop [6,7]. This has led to P enrichment of the top soil of agricultural lands [8,9] as P is almost immobile in soil. The residual P however, can gradually be available to crop depending upon soil type and soil properties [10], weather condition [4], cropping system and management practices.

Phosphorus in soil is present in both inorganic and organic forms. The inorganic P constitutes a major part of around 58-84% of total P present in soil [11] and comprises of many forms which remain in association with various mineral elements like Al, Fe, Ca and in many physical positions like occluded P and reductant P which unequally contribute to P nutrition depending on the soil and plant environment. In soils, rice crop is grown mostly under submerged condition where there is transformation of P fraction from one form to other depending on soil characteristics which are also influenced by the management practices including application of fertilizers and manure's [12]. In acidic soils, P is mainly sorbed to iron (Fe) and aluminum (Al) oxides and hydroxides, and in calcareous soils to calcium (Ca) carbonates [10]. For optimizing P management in crops in a particular agro ecosystem, it is very important to characterize the residual P remaining in the soil after repeated fertilizer P application. Long term field experiments are essential for providing key information on the impacts of management practices in soil and for assessing the sustainability of agro-ecosystem [13]. However, limited long term experimental work has been done in relation to the impacts of fertilization on soil P-changes and its dynamics in low land paddy fields.

Information on the composition of all chemical forms of P is fundamental to understanding of P dynamics and interactions in paddy soils, which in turn are necessary for its effective management. However, little is known about the cycling of P in soil, including the fate of P in long term fertilizer and manure applied to lowland paddy soils. Therefore, this paper aims to evaluate the effect of 13 years of Fertilizer and manure application on the transformation of the inorganic P fractions in intensively cultivated lowland paddy soil and their relative contribution to P nutrition using a long term fertilizer experiment with rice-rice production system under subtropical climatic situation of coastal Odisha.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted in the experimental field of All India Coordinated Research Project (AICRP) on Long Term Fertilizer Experiment (LTFE) of ICAR at OUAT, Bhubaneswar, India (20°17′ N, 85°49′ E and 30 m above mean sea level) which was started during 2005-06. The location of the experimental site is characterized as sub-humid subtropical climate with dry season from October to June and wet season from July to September. The average annual rainfall is 1453 mm, and the mean maximum and minimum temperatures are 31.40^{o}C and 21.10^{o}C, respectively. The experimental soil is a pale yellow (10YR6/8), lateritic *Inceptisol* (*UdicUstochrept*). The initial soil properties of 0-15cm layer were pH 5.3, bulk density 1.55 gcm^{-3}, cation exchange capacity 3.75 cmol(+)kg^{-1}, soil organic carbon (SOC) 4.4 g kg^{-1} , total P 632 kgha^{-1}, and available (Olsen) P 19.7 kgha^{-1}.

2.2 Experimental Details

The experiment consisted of 12 fertilizer treatments *viz*.,T_1=100%PK,T_2=100%NPK, T_3=150%NPK,T_4=100%NPK+Zn,T_5=100%NPK+ FYM,T_6=100%NPK+Lime+FYM,T_7=100%NPK+B +Zn,T_8=100%NPK+S+Zn,T_9=100%N,T_{10}=100%N P, T_{11}=100%NPK+Lime and T_{12}=Control, where 100% NPK correspond to 80-40-60 kg of N, P_2O_5 and K_2O ha^{-1}.The experiment was laid out in randomized block design (RBD) with four replications. Rice cultivar Swarna (MTU 7029) was grown in wet season and Lalat in dry season of every year. Twenty five days old rice seedlings were transplanted at a spacing of 20 cm × 10 cm with 2-3 seedlings per hill to puddled field in both the seasons. Nitrogen (N) was applied in three splits i.e. 25% at puddling as basal, 50% topdressing at 18 days after transplanting and 25% topdressing at panicle initiation stage. Entire dose of phosphorus (P) was applied during puddling as basal and potassium (K) was applied in two splits, 50% at puddling as basal and 50% topdressing at panicle initiation (PI) stage. Entire FYM (5 t ha^{-1}season^{-1}) was applied at the time of puddling. From the treatments the doses of NPK are clear as 100%NPK has been defined. FYM has been added @ 5 t ha^{-1} in each season in T_5 and T_6. Lime @ 1 t ha^{-1} in each season has been applied in T_6 and T_{11} at the time of land preparation. Zn has been applied as Zinc oxide @ 0.4% solution in T_4, T_7 and T_8. Borax was foliar sprayed twice as a source of boron @ 0.25% solution in T_7. Gypsum was applied to supply sulphur @ 30 kg ha^{-1} in T_8. Necessary uniform intercultural, water management and plant protection measures were undertaken in general until the crop was matured for harvesting. For the research work, intensive study was conducted on crops of two season dry, 2017-18 and wet, 2018 where 2 different rice varieties Lalat (120 days duration) and Swarna (145 days duration) were grown. Before harvest of crop grain yield was monitored through crop cutting.

2.3 Biomass Yield and P Content in Biomass

After harvesting in both the seasons, biomass yield and phosphorus content in both grain and straw was determined. Both grain and straw yields were estimated after weighing of air dried sample and making adjustment for moisture content. Laboratory analysis for P content was done after drying the sample at 70^{o}C in oven for 72 hours. The dried plant sample was digested in di-acid for determination of phosphorus content [14].

For determination of P content in gain and straw, plant sample (1g) was poured to 10ml HNO_3 taken in a 150 ml conical flask, which was kept overnight for pre digestion and then heat digested the next day on a hot plate for which 10ml of di-acid was added and gradually heated until just 2-3ml of samples remained in the flask. The flask was rinsed with distilled water, and the solution was filtered into a 100ml of volumetric flask then distilled water used for volume makeup. Vanado-molybdo-phosphoric acid reagent was used to estimate total P of soil /grain/straw, 5ml aliquot of 25ml volumetric flask, and the strength of yellow colour (Vanado-molybdo-phosphoric) was measured at 470nm in a spectrometer and content of P was estimated. Total phosphorous removed by the crop (grain and straw) in both the seasons was calculated separately and then total P removed from the soil in the year was calculated.

2.4 Soil Analysis

Total P in soil was also determined following the method similar to plant P estimation outlined by Jackson[14].Besides total P, fractions of inorganic P (Saloid-P, Fe-P, Al-P, Ca-P, Reductant Soluble–P and Occluded bound-P), were determined directly by sequential extraction method given by Peterson and Corey [15] modified by Kuo [16].

2.5 Extraction of P

2.5.1 Saloid bound phosphorous (Sal-P)

1g of soil was placed in a 50 ml centrifuge tube, 25ml of 1*M* NH_4Cl solution was added, and shaken for 30 minutes and centrifuged for 10 minutes @ 2000rpm. Aliquot from the supernatant solution was taken for spectroscopic measurement.

2.5.2 Aluminium bound phosphorous (Al-P)

The residue left was shaken for an hour with 25ml of NH_4F solution (pH 8.2) and then centrifuged. The residue was washed twice with 12.5 ml of saturated NaCl solution, centrifuged each time to recover the soil. The washing was discarded.

2.5.3 Iron bound phosphorus (Fe-P)

The soil was shaken for 17 hours with 50ml of 0.1*M* NaOH solution and then centrifuged. In the supernatant solution, concentrated H_2SO_4 was added until the organic colloids begin to flocculate and the suspension was taken for measurement. The precipitate was washed twice in saturated NaCl and the washings were discarded.

2.5.4 Reductant soluble phosphorus (Red-P)

After that, the soil was suspended in 25ml of 0.3*M* sodium citrate solution and 0.3g of sodium di-thionate was added, and the mixture was agitated for 10 minutes. The solution was heated in a hot water bath to 80°C, diluted to 50 mL, agitated for 5 minutes, and centrifuged. Excess citrate and dithionate were oxidised with 0.25 M $KMnO_4$.

2.5.5 Occluded bound phosphorous (Occl-P)

The residue was washed again, and the washing was disposed in the same manner as before. Shaking the soil with 0.1*M* NaOH for an hour then centrifuged @ 2000 rpm for 15 minutes.

2.5.6 Calcium bound phosphorus (Ca-P)

The remaining residue was washed twice more with saturated NaCl. Ca-P was collected by shaking the soil for an hour with 0.5ml of 0.25 *M* H_2SO_4.Total organic P was indirectly determined from difference between total P and inorganic P.

2.6 Estimation

5ml of the extracted aliquot was taken in 25ml of volumetric flask to which 2-3 drops of the p-nitrophenol indicator were added and the pH adjusted with 2 *M* H_2SO_4 until the indicator colour just changed and volume makeup by distilled water. Phosphorus concentrations were estimated using the ascorbic acid method at 880nm [17].

The amount of P in each fraction was calculated using the following equation:

P concentration in given fraction (mg kg^{-1}) = [Conc. of P (mg L^{-1})] x [R factor] x [Volume of extractant (L) ÷ mass of soil (kg)]

The total organic P was indirectly determined by from difference between total P and inorganic P.

Total organic P (kg ha^{-1}) = Total P ($kgha^{-1}$) – Total inorganic P ($kgha^{-1}$)

Total inorganic P ($kgha^{-1}$) = Sal-P+ Fe-P +Al-P +Red-P +Occl-P +Ca-P

Besides total and inorganic fractions of P other physio-chemical parameters like Olsen's P, pH, SOC, CEC, and Clay % were also studied by standard methods [14].

2.7 Statistical Analysis

Two –way analysis of variance was carried out to that effect of various treatments on the studied variables. DMRT was performed to find out the significance of pairwise mean difference among various treatments. Karl Pearson's product moment correlation analysis was carried out to find out the relationship between various parameters soil P fractions with grain yield, P uptake and soil properties. The correlation coefficients were tested at 1% and 5% level of significance. A multiple linear regression analysis was carried out to establish underline relationship between P uptake (dependent variable) and various P fractions (Independent variables).

$$\mathbf{U = b_o + b_1\,Sal.P + b_2\,Al.P + b_3\,Fe.P + b_4\,Red.P + b_5\,Occl.P + b_6\,Ca.P}$$

Where, **U** stand for phosphorus uptake , **Sal.P** stand for Saloid bound phosphorus, **Al.P** stand for Aluminium bound phosphorus, **Fe.P** stand for Iron bound phosphorus, **Red.P** stands for

Reductant bound phosphorus, **Occl.P** stands for Occluded bound phosphorus, and **Ca.P** stand for Calcium bound phosphorus.

Furthermore, a Hierarchical partitioning analysis [18, 19] was carried out to find out relative importance of various P fractions on uptake. All the statistical analysis was carried out using R statistical package.

3. RESULTS AND DISCUSSION

3.1 Grain Yield

3.1.1 Dry season, 2017-18

Result on grain yield of dry season 2017-18 showed [Table 1. ; Fig.1.(a-c)] that highest yield of 4453 kg ha^{-1} was produced in 100%NPK+Lime+FYM treatment which was at par with 100%NPK+FYM(4347 $kgha^{-1}$) demonstrating no effect of lime in presence of FYM applied @ 5 t ha^{-1}. But in absence of FYM, liming has significant effect (3525 kg ha^{-1}). P or K also significantly increased the grain yield. The soil of experimental plot has medium P availability. Further, during dry season, P availability was less as it diffused slowly at low temperature. So, application of P fertilizer in dry season significantly increased the grain yield. The soil has low available K (43.4 kg ha^{-1}) and within 13 yrs there is depletion of total K, so there is significant response to application of K particularly in dry season, as K availability in relatively less in dry season due to slow diffusion. Application of 50% more NPK also was effective in producing 13.26% significantly higher yield than 100%NPK and zinc did not produce any significant effect over 100%NPK. There was no significant response to application of B and S. Grain yield was significantly lower in control plot (1325 kg ha^{-1}) than all other treatments except 100%N that also produced vary low yield of 1751 kg ha^{-1}.

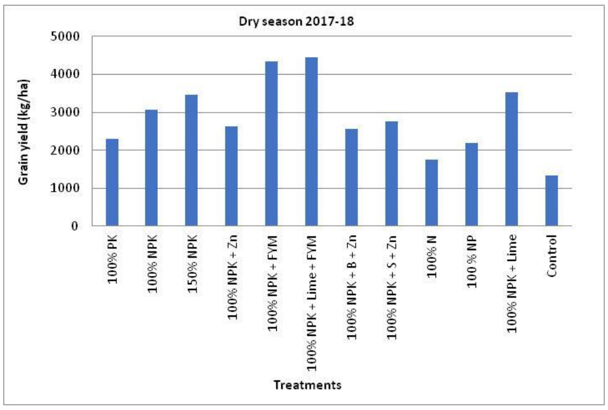

Fig. 1 (a).

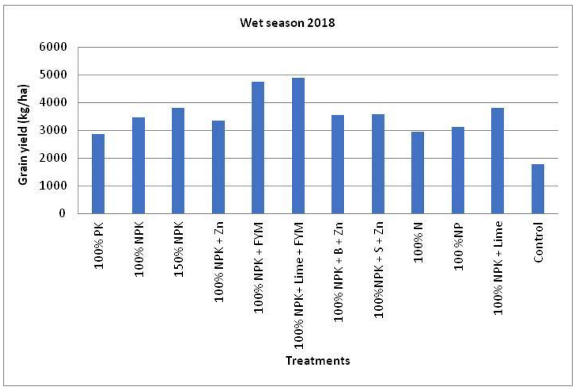

Fig. 1 (b).

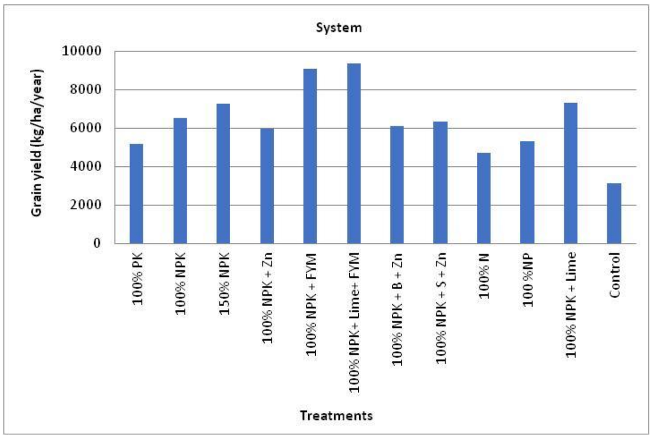

Fig. 1 (c).

Fig. 1. Grain yield of both the season dry (a) and wet (b) and total yield per year (c) under various manurial practice

Table 1. Long term effect of 13 years of cropping and manorial treatments on grain yield, P uptake and post harvest Olsen 'P

Treatments	Grain yield (kg ha^{-1})		P uptake (kg ha^{-1})		Olsen's P (kg ha^{-1})
	Dry season 2017-18	Wet season 2018	Dry season 2017-18	Wet season 2018	Post-harvest surface soil
100% PK	2291de	2873^{e}	7.66^{ef}	8.52^{e}	8.27^{b}
100% NPK	3063^{bc}	3456^{bc}	10.01^{cd}	11.97^{cd}	9.24^{b}
150% NPK	3469^{b}	3803^{b}	12.44^{b}	14.78^{b}	11.16^{b}
100% NPK + Zn	2625^{cde}	3353^{bcd}	8.66^{de}	11.14^{b}	9.85^{b}
100% NPK + FYM	4347^{a}	4750^{a}	16.62^{a}	19.79^{a}	49.72^{a}
100% NPK + Lime + FYM	4453^{a}	4891^{a}	16.89^{a}	20.55^{a}	53.41^{a}
100% NPK + B + Zn	2560de	3556^{bc}	8.90^{e}	12.42^{cd}	9.10^{b}
100% NPK + S + Zn	2763^{cde}	3582^{bc}	10.20^{cd}	11.43^{d}	9.82^{b}
100% N	1751^{fg}	2970de	5.08^{gh}	7.15^{e}	7.89^{b}
100 % NP	2186^{ef}	3132^{cde}	6.75^{fg}	8.83^{e}	8.84^{b}
100% NPK + Lime	3525^{b}	3807^{b}	11.61^{bc}	13.58^{bc}	10.59^{b}
Control	1325^{g}	1777^{f}	4.00^{h}	3.87^{f}	7.90^{b}
Initial					19.14

LSD (p<0.05%): in each coloum the values (mean of four replicates observations) followed by common letters are not significantly different (p, 0.05%) between treatments by DMRT

3.1.2 Wet season, 2018

The data revealed that the yield of wet season 2018 (Table 1.; Fig.1.b) rice (cv. Swarna) varied from a minimum of 1777 kg ha^{-1} recorded in control to a maximum of 4891 kg ha^{-1} in 100% NPK+FYM+Lime treatment. Application of FYM @ 5 t ha^{-1} was very effective in significantly increasing (37.44% rise) in the grain yield over 100%NPK, whereas lime @ 1 t ha^{-1} had no effect when applied along with FYM. Application of 50% more NPK also was effective in producing 10.04% significantly higher yield than 100%NPK. Application of zinc did not produce any significant effect over 100%NPK. Further, in the present investigation, application of B and S did not produce any significant effect over 100%NPK+Zn. Working on similar soil Majhi et al, [20] also found that continuous addition or exclusion of some secondary (S) and micro-nutrients (Zn and B) did not make any significant difference on grain yield. From the results it is also clear that FYM has significant effect on grain yield in both the seasons.

Combination of FYM+Lime to 100% NPK resulted in significant increase in grain yield in both the seasons in all the years. However, the response to FYM was more in wet season than in dry season. Higher response to FYM in the wet season than dry season has also been reported by Majhi and Rout [21]; Shahid et al [22]. Yield increased in 150%NPK than 100%NPK+FYM. Similarly, Srilatha et al. [23] also reported 22.10% more yield with 150% NPK than 100%NPK+FYM in a study conducted on *typic Ustochrept* on a clayey soil of Andhra Pradesh.

3.2 Phosphorus (P) –Uptake

The crop yield and uptake of nutrients are interdependent. The total uptake of nutrients for rice was calculated by adding the nutrient uptake [Table 1; Fig. 2.(a-c)] by both grain and straw biomass of individual season. Results pertaining to total P uptake of dry season 2017-18 and wet season 2018 and are presented in Table 1.Fig.2.(a and b). Total P uptake in dry season (2017-18) varied from 4.00 kg ha^{-1} to 16.89 kg ha^{-1}and in wet season 2018 varied from 3.87 kg ha^{-1} in control to 20.55 kg ha^{-1} in 100%NPK+Lime+FYM. 100%NPK+FYM was at par with 100%NPK+Lime+FYM as presented in Table.1. FYM amended plots resulted in more P uptake by releasing the organic acids during its decomposition [24] P uptake was more in all P treated plots than P minus treatments. Significantly higher uptake was recorded with super optimal dose (150%NPK) than 100%NPK. No significant effect was observed for secondary nutrient (S) and micronutrients (Zn and B) applied with optimal dose. Uptake of nutrients was lower in the control plot due to absence of external source of nutrient to the plants [25].

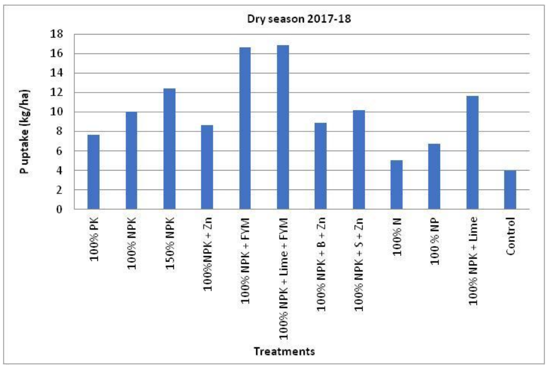

Fig. 2 (a).

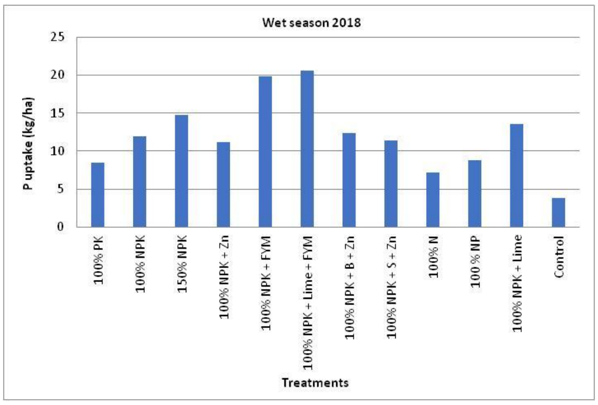

Fig. 2 (b).

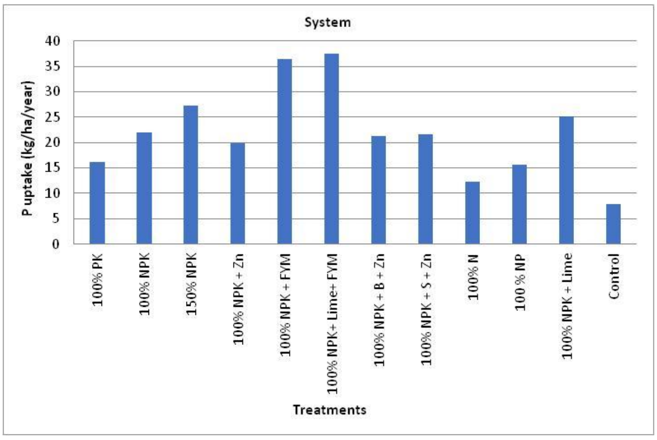

Fig. 2 (c).

Fig. 2. P uptake of rice in both the season dry (a), wet (b) and system P uptake/year (c) under various manurial practice

3.3 Available Phosphorus (Olsen's P)

Results on available P (Olsen's P) presented in Table1. reveal that continuous application of FYM along with optimal level of NPK either with or without lime maintained significantly higher quantity of Olsen's P than non FYM and non-lime treatments. Highest available P (53.41 kg ha^{-1}) was measured in NPK+FYM+Lime treatment which was at par with NPK+FYM treatment (49.72 kg ha^{-1}). P content increase in pH with application of FYM and lime that favored P desorption from clay, iron and aluminum oxides. Such pH changes favoured desorption of freshly applied P only [26].

3.4 Changes in P Fractions

Data pertaining to various P fractions recorded in Table 2. reveal that after 13 years of continuous cropping the total inorganic P (Pi) varied from 337.73 kg ha^{-1} in control plot to a highest of 684.96 kg ha^{-1} in 150%NPK treatment and organic P (Po) varied from a lowest of 48.58 kg ha^{-1} in 100%N plot to a highest of 204.29 kg ha^{-1} in 100% NPK+FYM treatment .The Pi fraction constituted 64.43-89.60% where as Po fraction 10.40-35.57% of the total phosphorus. The inorganic fractions on an average thus constituted 76.46 % and organic P, 23.53 % of the total P under the rice–rice ecosystem of eastern India (Fig.3). The organic P constituted one third of the total inorganic P in the intensively rice grown acid soil of subtropical condition. In a study under similar situation Sanyal et al, [11] reported total inorganic phosphorus (Sal-P, Fe-P, Al-P, Red-P, Occl-P and Ca-P) constituting 58-84% of total P present in soil. The lowest value of all inorganic fraction of P observed under control i.e. cultivation without fertilizers might be due to continuous removal of P from soil P reserve without any replenishment through fertilizers [25].

Table 2. Effect of long term manurial practices on different phosphorus fraction of surface soil (0-15 cm)

Treatments	Sal-P (kg ha^{-1})	Fe-P (kg ha^{-1})	Al- P (kg ha^{-1})	Red-P (kg ha^{-1})	Occl-P (kg ha^{-1})	Ca-P (kg ha^{-1})	Total Pi (kg ha^{-1})	Total Po (kg ha^{-1})	Total P (kg ha^{-1})
100% PK	43.34^{bc}	251.21^{b}	58.85^{b}	150.36^{abcd}	48.67^{def}	47.50^{c}	599.93^{bcd}	199.08^{ab}	799.02^{a}
100% NPK	40.85^{cd}	238.00^{bc}	56.17^{b}	151.93^{a}	46.91^{ef}	51.89^{bc}	585.74^{cde}	181.62^{ab}	767.37^{ab}
150% NPK	48.57^{b}	288.38^{a}	76.19^{a}	152.48^{a}	62.50^{bc}	56.84^{b}	684.96^{a}	156.96^{ab}	841.92^{a}
100%NPK + Zn	44.80^{bc}	270.16^{a}	54.56^{b}	150.83^{a}	60.96^{bc}	54.44^{b}	635.75^{ab}	169.35^{ab}	805.10^{a}
100% NPK + FYM	62.98^{a}	170.04^{cd}	55.93^{b}	108.71^{bc}	77.86^{a}	85.36^{a}	560.87^{cde}	204.29^{a}	765.17^{ab}
100% NPK+ Lime+ FYM	57.44^{a}	171.56^{cd}	61.75^{a}	104.69^{c}	68.75^{ab}	89.39^{a}	533.57^{de}	182.11^{ab}	735.69^{abc}
100% NPK+B+Zn	43.75^{bc}	234.79^{bc}	57.05^{b}	150.65^{a}	58.33^{cd}	54.63^{b}	612.40^{bc}	151.35^{ab}	763.75^{ab}
100%NPK+S+Zn	37.19^{de}	239.36^{bc}	48.97^{c}	145.19^{a}	50.72^{de}	67.79^{c}	605.21^{cde}	167.02^{ab}	772.24^{ab}
100% N	27.16^{f}	174.98^{cd}	39.53^{d}	103.66^{c}	36.64^{g}	36.58^{d}	418.55^{f}	48.58^{b}	467.13^{c}
100 %NP	32.23^{ef}	239.43^{bc}	45.16^{c}	129.49^{ab}	40.53^{fg}	40.70^{d}	527.53^{e}	200.08^{ab}	727.62^{abc}
100% NPK+lime	46.91^{bc}	193.61^{bcd}	46.54^{c}	137.11^{a}	48.83^{def}	75.28^{a}	548.28^{cde}	201.95^{ab}	750.19^{abc}
Control	20.35^{g}	152.89^{d}	35.07^{d}	79.36^{d}	25.19^{h}	24.87^{e}	337.73^{g}	186.45^{ab}	524.18^{bc}
Initial	22.35	172.0	50.62	85.50	28.31	24.87	383.65	248.35	632

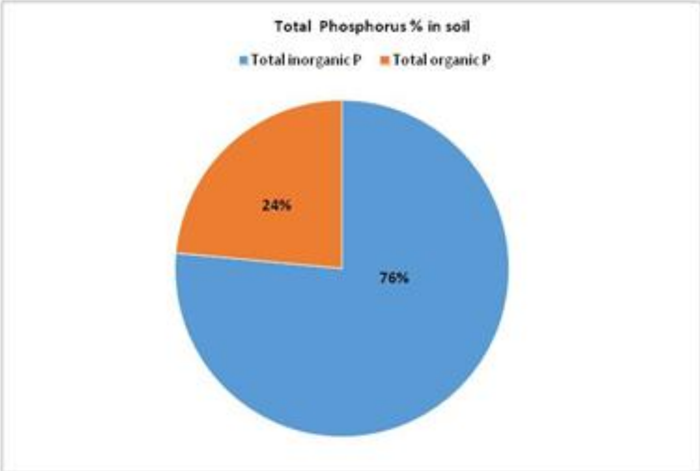

Fig. 3. Percentage distribution of organic and inorganic P fractions in surface soil

After 13 years Sal-P ranged from 20.35 to 62.98 kg ha^{-1}, Fe-P 152.89 to 288.38 kg ha^{-1}, Al-P, 35.07 to 61.75 kg ha^{-1}, Ca-P, 24.87 to 89.32 $kgha^{-1}$, Red-P 79-152 kg ha^{-1}, Occl–P 25.19 to 77.86 kg ha^{-1}. From the result it is observed that the content of Sal-P is the least among all the fractions which has also been reported by many workers [27,28]. The fractions in order of their content are: Fe-P > Red-P > Ca-P > Al-P > Occl-P > Sal-P, in contrast to the trend of P fraction, Fe-P > Red-P > Al-P > Ca-P > Occl-P > Sal-P measured in an *Inceptisol* [29]. The increase of Fe-P than Al-P due to the availability of NaOH extractable inorganic P serves as primary sink for applied P fertilizer in tropical soil and also in other cropping systems in various soils under continuous cropping system [30]. After 13years of continuous cropping the available P(Olsen's P) ranged between 7.89 and 53.41 kg ha^{-1} as compared to the initial status of 19.14 kg ha^{-1}.

The fractions of inorganic P in soil and its availability thus varied to a great extent due to different long term manorial management practices followed on a particular soil and climatic situation.

3.5 Long term treatment effects on various P fractions in an acidic *Inceptisol* under wetland rice-rice system

3.5.1 Saloid bound phosphorus (Sal-P)

Sal-P is the smallest fraction among all inorganic P fractions in surface soil. It constituted 6.13-11.31% of total inorganic P and 3.88-8.23% of total P. After 13 years of continuous cropping, comparison of means of major effect of different manorial treatments on Sal–P (Table 2; Fig.4.) showed highest Sal-P in 100% NPK+FYM (62.98 kg ha^{-1}) and lowest in 100%N (27.16 kg ha^{-1}) as compared to the initial status of 22.35 $kgha^{-1}$ which is 5.83% of total inorganic P and 3.54% of total P present in soil. This increase in Sal-P concentration was more with the combined application of organic manure than inorganic fertilizers alone due to release of P from organic matter through mineralization [31]. Application of FYM@ 5 t $ha^{-1}season^{-1}$ increased the Sal-P by 54.17% in absence of lime and 34.26% in presence of lime applied @ 1 t ha^{-1} indicating negative interaction effect of lime and FYM on Sal-P. Application of Zn @ 0.4% ZnO root dip also increased Sal-P by 9.67%. Zinc and phosphorus interaction lead to highly significant increase in the uptake of phosphorus and zinc in wheat and rice crops [32, 33]. Combination of S or B with Zn however, in our study had negative effect on Sal-P. In contrast, both N and K had positive effect on Sal-P. Sulphur has been applied through gypsum @ 250 $kgha^{-1}season^{-1}$ which contain both Ca and S. Application of gypsum increase the anionic strength of sulphur [34; 35] which promotes flocculation of smaller soil particles and adsorption of anions become strong resulting in less P in soil solution (Sal-P). With increase in Zn concentration in soil there is increase in acid phosphate activity which causes more mineralization of organic P and release of P into solution. But, foliar application of B has almost no direct significant effect on soil concentration of P. The slight reduction in Sal-P might be due to more uptake of P from root rhizosphere with foliar spray of B.

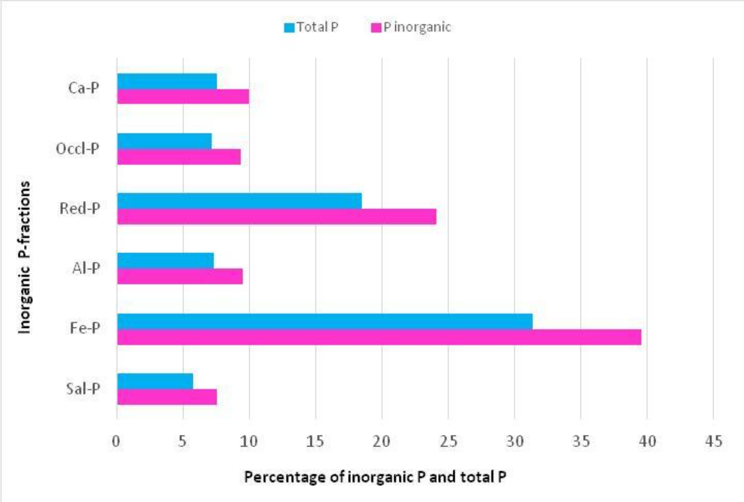

Fig. 4. Composition of various inorganic fractions of phosphorus as percentage of total inorganic P and total P in surface soil

Sal-P refers to the water soluble and freely exchangeable P in soil. Application of N stimulates organic anion produce from SOM decomposition and release of more P into the system leading to increase in Sal- P [36].

Thirteen years of continuous application of N and K caused 33.46% and 26.74% increase in Sal-P respectively. Similar effect of N and K was also reported by Kaur [37].

3.5.2 Iron bound phosphorus (Fe-P)

Results on Fe-P content of surface soil measured after 13 years of cropping (Table 2) revealed that it is the largest fraction among all the inorganic fractions of P constituting 30.53-45.75 % of total inorganic P and 22.22-37.46% of total P in soil. The results of comparison of mean values and the ANOVA test showed that the treatments had significant effect on Fe-P content under the prevailing soil situation. There was significant increase in its content in all fertilized treatments except 100%N, 100%NPK+FYM and 100% NPK+FYM+Lime treatments where the initial content (172 kg ha^{-1}) remained almost unchanged. P added through mineral fertilizer to acidic soils gradually reacts with Fe and Al-P compound and is transformed into relatively insoluble P compounds [38]. The treatment 150%NPK registered highest Fe-P of 288 kg ha^{-1} which was 21.01 % more than the optimum level (100%NPK), 88% more than control (152.89 kg ha^{-1}) and 67.66% over the initial 172 kgha^{-1}. Combination of FYM (5 t ha^{-1}), however, resulted in decrease in Fe-P content by 28.55% over 100%NPK. Organic matter in soil causes ferric iron reduction through its promoting influence on the bacterial activity in flooded soil. Humus and humus forming materials also help to decrease the fixation of P ions on Fe and Al oxides and maintain a steady availability of P to plants [39]. Application of compost decreased the residual P and Fe-P fraction in acid soil [40].

3.5.3 Aluminum bound phosphorus (Al-P)

Results on Al-P content of surface soil measured after 13years of cropping (Table 2.) revealed that it is the 4th largest fraction among all the inorganic fractions of P that varied from 35.07 kg ha^{-1} in control to a highest of 61.75 kg ha^{-1} in 150%NPK treatment constituting 8.36-11.15 % of total inorganic P and 6.20-9.05% with total P in soil. The results of comparison of mean values and the ANOVA test showed that the treatments had significant effect on Al-P content under the prevailing soil situation. Continuous application of N fertilizer on rice field increased the Al-P content by 12.71% over control. Potassium and zinc increased the Al-P content by 24.37% and 2.86% respectively over 100%NPK. In contrast, secondary and micro nutrients (S and B) had negative impact on Al-P availability in rice soil. Continuous application of lime also caused reduction in Al-P content.

It is interesting to note that unlike Fe-P, Al-P was not influenced by application of FYM. In a laboratory study Kamar et al, [41] however, showed that FYM @ 20 t ha^{-1} significantly increased Saloid-P, Al-P and Fe-P but showed non-significant increase in Ca-P [41].

The Al-P and Fe-P pools are expected to have very low bio-availability but can be used by plants when available soil P is severely low [42].In acidic soils, the original superficial, loosely bound phosphates to Fe and Al-oxides available to plant are converted gradually via a re-precipitation process into highly crystalline Fe and Al-P(not available to plant) [40].

3.5.4 Calcium bound phosphorus (Ca-P)

Ca-P varied from a lowest of 24.87 kg ha^{-1} in control constituting 7.36% total inorganic P to a highest of 89.39 kg ha^{-1} in 100%NPK+FYM+Lime treatment constituting 16.15% and 12.15% of total inorganic P and total P. Application FYM and lime significantly increased the Ca-P by 64.50% and 45.07% respectively over 100%NPK. There is Ca accumulation through addition of FYM (@32 kg Ca ha^{-1} yr^{-1}) and lime (400 Kg Ca ha^{-1} yr^{-1}) in the treatments where, FYM and Lime are added. Further there is also increase in pH which causes more Ca-P in these treatments [43,36]. Application of Zn as ZnO (0.4%) root dipping increased the Ca-P by 4.91% and secondary nutrient (S) and micro nutrient (B) increased the Ca-P content by 30.64% and 5.28% respectively. Addition of K increased the Ca-P by 27.49% while continuous application of N alone in form of urea increased Ca-P by 47.08%. Similar result was reported by Kaur [37].

3.5.5 Reductant soluble phosphorus (Red–P)

The Red-P and Occl- P are dominant in acid red laterite soils than neutral-alkaline or black clay soil [44] or due to weathered condition of the soil studied in the soils of Karnataka, Tamil Nadu, Gujarat and AP [45]. The results of ANOVA test showed that the treatments had significant effect on Red-P after 13 years of cropping. Red-P varied from lowest of 79 kgha^{-1} in control plot to a highest of 152.48 kg ha^{-1} in 150%NPK treatment. Application of graded dose of NPK fertilizer caused an increase in Red- P over control [46].

Within 13 years, there was increase in Red-P in all fertilized treatments over the initial status, 85.50 kg ha^{-1}whereas, there was significant decrease in control. With application of organic manure, @ 5 t ha^{-1}season^{-1} there was significant decrease in Red-P as compared to 100% NPK because FYM chemicals bind the Red-P strongly [46]. There was decrease by 28.44% to 31.09 % in FYM and FYM + Lime amended treatments indicating lesser accumulation in less active form of P in soil. Contrary to this, there is report that increase in the addition of organic fertilizer will enhance the Red-P fraction in acid soil [47].

With application of lime @ 1 t $CaCO_3$ ha^{-1}, Red-P also decreased to a greater extent in absence of FYM. Application of Zn as 0.4% ZnO solution root dipping, there was slight decrease (0.72%) in Red-P. Conjoint application of B or S with Zn however further reduce the Red-P by 0.84%, 4.43% respectively indicating formation of more less active form. Application of P or K also had significant effect on built up of this less active / recalcitrant form of P on the surface soil. Continuous application of N enhanced the Red-P bound P by 30.62 %. Amount of Red-P in surface soils could be attributed to the weathering of soil.

3.5.6 Occluded bound phosphorus (Occl-P)

Occl- P is another less active fraction of P in soil which constituted 7.74-13.98 % of inorganic P and 4.81-10.18% of total P with lowest found with control and highest with 100%NPK+FYM in acid soil. After 13 years of continuous cropping and manuring there was increase in Occl- P in all P fertilized treatments with highest recorded in 100% NPK +FYM (77.86 kg ha^{-1})and lowest in 100% NP treatment (40.53kg ha^{-1}) as compared to the initial status of 28.31 kg ha^{-1}. Continuous application of lime with FYM (0.34%) and lime alone (4.09%) have much effect in increasing the Occl-P. Amount of occluded Fe-P/Al-P could be attributed to the weathering of the soils. Addition of K and continuous application of N increased Occl-P by 15.74% and 45.45% respectively. Super optimal dose of P enhanced the occluded P by 33.23%. Higher accumulation in 150%NPK and relatively lower occluded P in high yielding treatments suggest that P bound to Occl-P is used more by rice through higher biomass production in high yielding treatments *viz.* 100%NPK+FYM and 100%NPK +FYM + Lime.

3.5.7 Total Phosphorus (Total P)

Data on changes in total P of surface soil layer (0-15cm) (Table 2.) show that there is accumulation of total P in all P treated soils and depletion in P minus treatments. Within 13 years of continuous cropping without fertilizer resulted in a decrease of 108 kg P ha^{-1} or 17.08% from the initial 632 kg ha^{-1}. Among the P applied treatments, highest accumulation(841.92 kg ha^{-1})

was found with the super optimal dose (150%NPK) which was 9.77% more than that with optimal dose (100%NPK). Total P content in high yielding treatments such as 100%NPK+FYM and 100%NPK+FYM+lime was less than that of 100%NPK treatment. Addition of recommended dose of K to 100%NP increased the total P by 5.35%.On the other hand, the total P content decreased with application of micro and secondary nutrients (Zn, S and B) in conjunction with 100% NPK. Less accumulation in high yielding treatments is due to more removal by the above ground crop biomass which is displaced from the field.

3.6 Inter Relationship of Seasonal Crop Yield (Dry and Wet) with P Uptake in Terms of Correlation Coefficient (R^2)

Correlation studies made on the crop yields and P uptake of both dry season (2017-2018) and wet season (2018) depicted in Fig. 5.(a to c) showed highly significant correlation between crop yields and P uptakes of both the seasons and system as a whole.

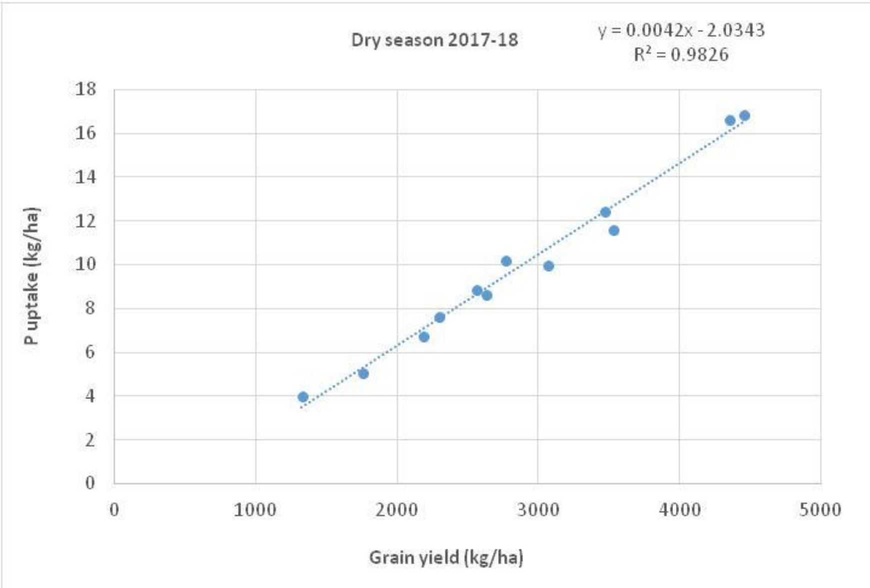

Fig. 5 (a). Interaction between dry season yield and dry season P uptake

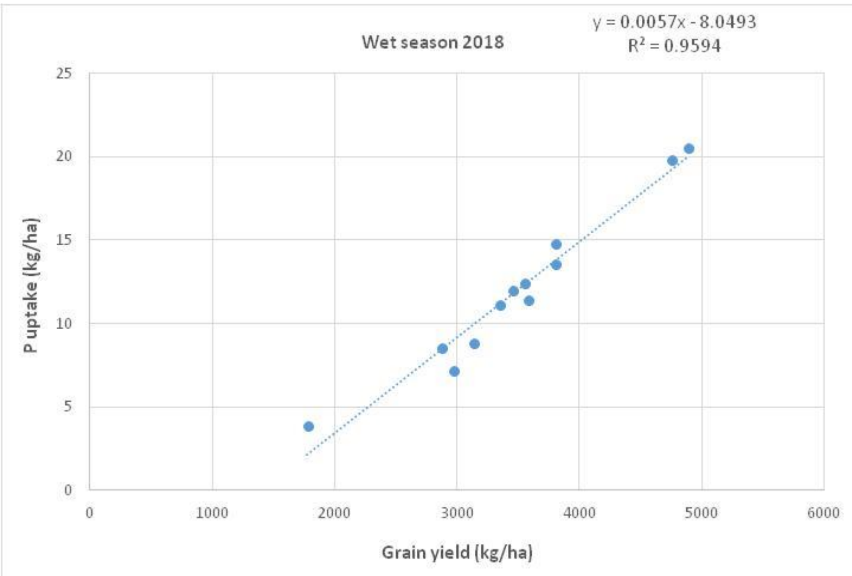

Fig. 5 (b). Interaction between wet season yield and wet season P uptake

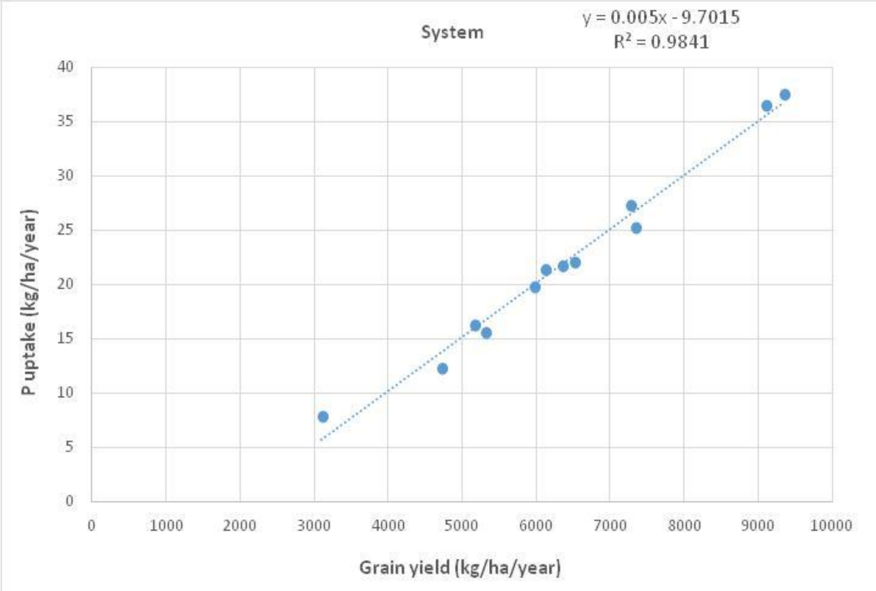

Fig. 5 (c). Interaction between system yield and system P uptake

Table 3. Correlation of various P fractions of surface soil with crop yield and P uptake expressed in term of correlation coefficient (r^2)

	Grain Yield		P uptake	
P-fractions	**Dry season 2017-18**	**Wet season 2018**	**Dry season 2017-18**	**Wet season 2018**
Sal-P	0.580***	0.642***	0.581***	0.640***
Fe-P	0.042^{NS}	- 0.058^{NS}	0.004^{NS}	-0.047^{NS}
Al-P	0.372**	0.413***	0.387**	0.457***
Red-P	0.079^{NS}	0.128^{NS}	0.079^{NS}	0.092^{NS}
Occl-P	0.659***	0.673***	0.697***	0.774***
Ca-P	0.0.819***	0.795***	0.810***	0.857***
Total P	0.394**	0.356*	0.375**	0.383**
Total Pi	0.395**	0.350*	0.373**	0.377**
Total Po	0.084^{NS}	0.083^{NS}	0.077^{NS}	0.093^{NS}

*Note: p >0.05 ' NS '(non significant), p<(0.05)= ' * ', p <(0.01) = ' **' and P<0.001 = ' ****

Relationship of various P fractions in soil with crop yield and P uptake of both the seasons was presented in Table 3. The results reveal that grain yield of rice in dry and wet season have highly significant correlation, with Sal-P (r= 0.580***, 0.642***), Al-P (0.372**, 0.413**) and Ca-P (0.819***, 0.795***) respectively. Similar result was reported by Kaur [37]. Among the various P fractions Ca-P show strongest positive correlation with both grain yield (r= 0.819***, 0.795***) and P uptake (r= 0.810*** , 0.857***)in both dry and wet season respectively followed by Occl-P (0.659***, 0.673***) , (0.697***, 0.774***) Sal-P (r= 0.580***, 0.642***), (0.581***, 0.640***), and then Al-P (0.372**, 0.413***) , (0.387**, 0.457***) respectively but, Fe-P Red-P and organic P had non-significant correlation.

3.7 Correlation of soil properties with various P fractions measured on post harvest surface soil of wet season 2018

Results of correlation between various fractions of soil P and soil properties (pH, SOC and CEC) (Table 4) reveal that among the fractions, Sal-P, Occl-P and Ca-P have significant positive correlation with pH, SOC and CEC, whereas, Fe-P and Red-P have negative correlation with pH and SOC. Total inorganic P, organic P and total P have non-significant correlation with pH and available P. CEC have strong correlation with inorganic P and total P, while SOC with total P only at 5% level of significance.

Table 4. Correlation of soil properties with various P fractions measured on postharvest surface soil of wet season 2018 expressed in term of correlation coefficient (r)

P-fractions	pH	SOC	CEC	Olsen's P
Sal-P	0.424**	0.594***	0.608***	0.469***
Fe-P	-0.290*	-0.155^{NS}	0.081^{NS}	-0.148^{NS}
Al-P	0.057^{NS}	0.374**	0.529***	0.318^{*}
Red-P	-0.179^{NS}	-0.117^{NS}	0.154^{NS}	-0.273^{NS}
Occl-P	0.391^{**}	0.485***	0.696***	0.639***
Ca-P	0.682***	0.604***	0.634***	0.739***
Total Pi	-0.070^{NS}	0.136^{NS}	0.472***	0.087^{NS}
Total Po	0.159^{NS}	0.266^{NS}	0.145^{NS}	-0.121^{NS}
Total P	0.087^{NS}	0.343*	0.485***	0.185^{NS}

Note: p>0.05 'NS'(non-significant), p< (0.05) = '' , p <(0.01) = '**' and p<0.001 = '***'*

3.8 Relative contribution of surface soil p fractions to P uptake by rice in different seasons

A multiple regression analysis was carried out taking dry season P uptake as dependent variable and P fractions as independent variable. The overall variation explained by all fractions was 69.75%. From the regression equation it is revealed that among the inorganic P fractions, the effect of Ca-P is highly significant on P uptake (Table 6.; Fig. 6.(a)). Furthermore, a relative importance analysis was carried out to find the independent effect percentage of each fraction on dry season P uptake. The results revealed that, Ca-P has maximum independent contribution to P uptake accounting for 45.41 % followed by Occl-P 25.86%, and then Sal-P 18.68% and Al-P 6.90% suggesting these four fractions to be the maximum contributing fractions on dry season P uptake variation. The fractions in order of their contributing importance are: Ca-P > Occl-P > Sal-P > Al-P > Fe-P > Red-P.

Results of multiple regression analysis carried with wet season P uptake showed that the overall variation explained by all fractions was 78.79%. From the regression equation it was revealed that the effect of Ca-P is highly significant on P uptake. Results of relative importance analysis carried out to find the independent effect percentage of each fraction on wet season P uptake showed that, Ca-P has maximum independent contribution accounting for 42.57% followed by occluded P 28.59% and then Sal-P 18.56% and Al-P 7.63% in wet season uptake variation (Table 6. and Fig. 6.(b)). show the percentage contribution of each fraction on wet season uptake. The regression equation obtained is shown in Table 5.

A multiple regression analysis carried out with various in organic P fractions of surface soil for annual total P uptake as independent variable showed (Table 6; Fig. 6(c)) that the overall variation explained by all fractions was 77.28%. From the regression equation it is revealed that the effect of Ca-P is highly significant on P uptake. Furthermore a relative importance analysis carried out to find the independent effect percentage of each fraction on P uptake revealed that, Ca-P has maximum independent contribution accounting for 43.90%, followed by Occl-P 27.39%, Sal-P 18.64% and Al-P 7.28% to system P uptake. In rice soil, there

Table 5. Regression equation representing contribution of P fractions as independent variable on uptake of phosphorous as dependent variable

	Regression equation of independent fractions and uptake as dependent factor
Dry season uptake	$U = -1.332 + 0.077P_{Sal} - 0.056P_{Al} + 0.002P_{Fe} + 0.016P_{Red} + 0.026P_{Occl} + 0.123P_{Ca}$
Wet season uptake	$U = -1.762 + 0.081P_{Sal} - 0.060P_{Al} - 0.004P_{Fe} + 0.021P_{Red} + 0.601P_{Occl} + 0.139P_{Ca}$
Total uptake	$\mathbf{U = -3.097 + 0.157P_{Sal} - 0.116P_{Al} + 0.002P_{Fe} + 0.037P_{Red} + 0.087P_{Occl} + 0.262P_{Ca}}$

Table 6. Relative contribution of inorganic P fractions to the total explained variation (%)

Variables	Relative Contribution of uptake on fractions		
	Dry season uptake	**Wet season uptake**	**Total uptake**
Sal-P	18.68	18.56	18.64
Fe-P	1.74	1.20	1.35
Al-P	6.90	7.63	7.28
Red-P	1.39	1.43	1.41
Occ-P	25.86	28.59	27.39
Ca-P	45.41	42.57	43.90
R^2	69.75	78.79	77.28

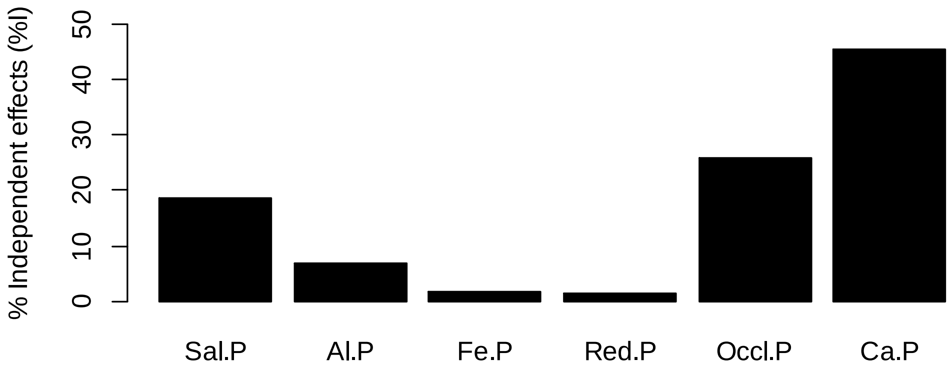

Fig. 6 (a). Percentage independent effect of fractions of P on dry season uptake

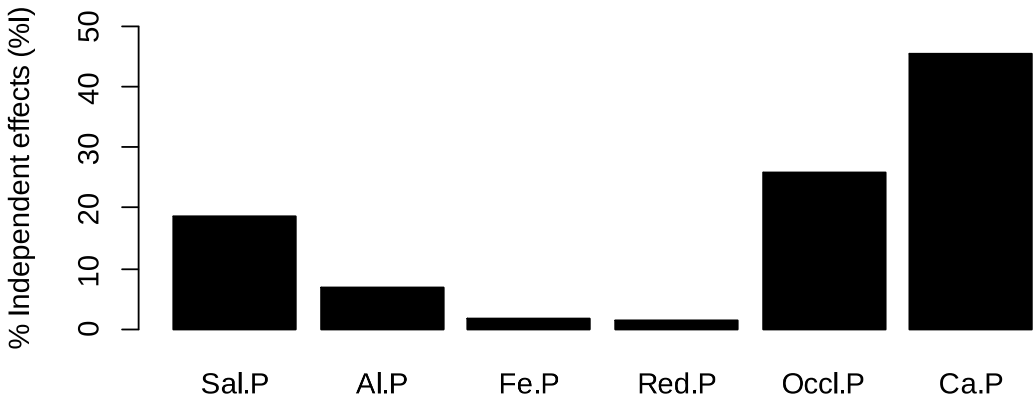

Fig. 6 (b). Percentage independent effect of fractions of P on system P uptake

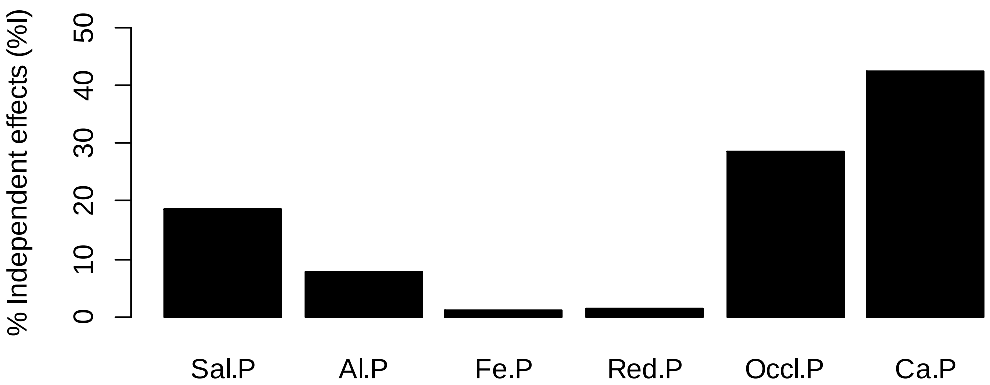

Fig. 6 (c). Percentage independent effect of fractions of P on total uptake

is good quantity of Ca-P which becomes labile with slight rise in pH of acid soil on submergence as compared to non labile form (Al/Fe oxide and hydroxides) of P present in acid soil [48,49]. The indirect and direct effect of occluded P as source of P on the available P pool for P nutrition can be attributed to the dissolution of the stable forms of inorganic P found as occluded P fraction [50].On the other hand, Reductant soluble P is considered to be recalcitrant and hence not extractable and is unavailable [51].

Thus it is inferred that among the total inorganic P fractions of surface soil, Ca-P contributes maximum to P uptake in both the seasons followed by occluded P then Sal-P and Al-P. Relative contribution of Fe-P and reductant P is minimum. The fractions in order of their relative importance for P uptake under submerged rice-rice situation are: Ca-P > Occl-P > Sal-P > Al-P > Red-P > Fe-P.

4. CONCLUSION

Combination of FYM and 100% NPK resulted in significant increase in grain yield in both dry and wet season. However the response to FYM was more in wet season than in dry season. Application of Zn or Zn+B or Zn+S did not have any significant impact on grain yield. Continuous application of FYM along with optimum level of NPK either with or without lime maintained significantly higher level of Olsen P than non FYM and non lime treatments. Results showed that there was build-up of total P in all P treated plots and depletion in P minus treatments. Highest accumulation of 841.92 kg ha^{-1} was found in super optimal dose (150%NPK) which was 9.77% more than the optimal dose (100%NPK) but total P content in high yielding treatment such as 100% NPK+FYM and 100% NPK+FYM+Lime was less than that of 100%NPK treatment. Continuous cropping without P resulted in decrease of total P in surface soil. In control the decrease was 17.08% from the initial 632 kg ha^{-1}. The inorganic P fraction on surface soil constituted 64.43-89.60% where as the organic P fraction 10.40-35.57% of total Phosphorus. Organic P thus constituted about $1/3^{rd}$ of total inorganic P in the rice-rice system of the eastern India. The inorganic fraction in terms of their abundance were in the order Sal-P (20.35-62.98 kg ha^{-1}) < Occl-P (25.19-77.86 kg ha^{-1})<Al-P (35.07-61.75 kg ha^{-1})<Ca-P (24.87-89.39 kgha^{-1}) < Red–P (79.36-167.83 kg ha^{-1}) < Fe-P (152.89-288.38 kg ha^{-1}). Olsen P which was significantly influenced by the fertilizer treatments had a very strong positive correlation ($p<0.01$) with Sal-P (r= 0.469***), Occl-P (r=0.639***) and Ca-P (r=0.739***). Among the inorganic P fractions, Ca-P contributed highest of 42.57% to P uptake, followed by Occl-P (28.59%), Sal-P (18.56%), and Al-P (7.63%) and lowest was contributed by Fe-P. Relative contribution of Fe-P and red-P is negligible. So, the fractions in order of their relative importance for P uptake under submerged rice-rice situation are: Ca-P > Occl-P > Sal-P > Al-P > Red-P > Fe-P.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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