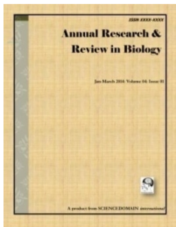

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Status of Soil Phosphorus in Context with Phosphate Solubilizing Microorganisms in Different Agricultural Amendments in Kachchh, Gujarat, Western India

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Authors' contributions

Author SBS designed the study, performed the experimental analysis and drafted the manuscript. Author RZS provided guidance and improved the manuscript. Author MHT provided guidance. Author GAT provided guidance. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The study was carried out to assess the soil Phosphorus (P) status (available and total) in farms with organic amendments and compared the results to those which practice an integrated amendment system. In conjugation to P status of soil, the soil microbial flora in terms of total microbial count and Phosphate Solubilizing Microorganisms (PSM) was studied. The soil chemical parameters such as pH, electrical conductivity (EC), available nitrogen and potassium were also evaluated.
Study Design: The sites selected for study comprised 8 fields of maize (*Zea mays*) on two farms (4 fields for A1 and 4 fields for A2).The organic amendment (A1) included farm yard compost (FYC) applied at the rate of 4 ton/Ha, as a basal dose which was applied before sowing. In these fields, after sowing, a five day fermented concoction of cow dung, jaggery, soil, cow urine and gram flour was applied with watering twice, at seven days

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interval from sowing. In the integrated amendment fields (A2) FYM (farm yard manure) at the rate of 1 ton/Ha) was applied as a basal dose before sowing and DAP (di ammonium phosphate) (40 Kg/Ha) + urea (60 Kg/Ha) was applied at 15-20 days after sowing. The study was carried out for two seasons of crops in the year of 2012, February-May and July-Oct , at three different phases of crop growth (15, 45 and 60 days after sowing).
Place of Study: Different agricultural fields in semi-arid zone of Kachchh, Western India (23º13'48.00''N, 69º42'35.06''E) were chosen.
Methodology: Samples were collected from the rhizosphere of the crop upto the depth of 12 cm using standard soil sampling procedure. Four samples were collected per hectare and pooled to form one composite sample. Samples were analyzed in triplicates. Soil samples were divided into two parts, one part of the sample was air dried and sieved through a 2 mm sieve and analyzed for chemical characteristics and another part of soil sample was stored at 4°C for microbiological analysis.
Results: The results demonstrated that although this zone of western India has good amounts of total P, the available P status was statistically significant ($P \le 0.05, 0.01, 0.001$) in organic amended fields than integrated fields. The organic amendments were able to maintain a good microbial count (total as well as PSM) than integrated fields. This confirms that efficient nutrient management in cropping systems could lead to build up of microbial population especially PSM over time which in turn solubilize the fixed P and make it available to plants.
Conclusion: Phosphorous is an important element after nitrogen as a mineral nutrient in terms of quantitative plant requirement. Although abundant in soils, in both organic and inorganic forms, its availability is restricted as it occurs mostly in insoluble forms. PSM play a vital role through solubilisation and mineralization of fixed P. The present study focused on highlighting the beneficial effects of organic farming practices over integrated amendments, especially in the semi-arid tropics.

Keywords: Soil phosphorus; PSM; P solubilization; fertilizers; organic; integrated amendments.

1. INTRODUCTION

Phosphorus (P) is the most important key element in the nutrition of plants, next only to nitrogen (N). It plays an important role in virtually all major metabolic processes in plants including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration [1] and promotes N fixation in legumes [2]. Although P is abundant in soils in both inorganic and organic forms, it is frequently a major limiting factor for plant growth as it is in an unavailable form for root uptake. The soils that exhibit highest P fixation capacity occupy 1,018 million hectares (Ha) in the tropics [3]. The term P fixation is used to describe reactions that remove available phosphate from the soil solution into the soil solid phase. To circumvent this deficiency, chemical P fertilizers are added which not only represent a major cost of agricultural production but also impose adverse environmental impacts on overall soil health and degradation of terrestrial, freshwater and marine resources [4]. The repeated and injudicious applications of chemical P fertilizers, lead to the loss of soil fertility [5] by disturbing microbial diversity, and consequently reducing yield of crops.

The efficiency of applied P fertilizers in chemical form rarely exceeds 30% due to its fixation, either in the form of iron/aluminium phosphate in acidic soils [6] or in the form of calcium phosphate in neutral to alkaline soils [7]. The realization of all the potential problems associated with chemical P fertilizers together with the enormous cost involved in their manufacture, has led to the search for environmentally compatible and economically feasible alternative strategies for improving crop production in low or P-deficient soils [8]. Microorganisms are an integral component of the soil P cycle and are important for the transfer of P between different pools of soil P. Phosphate Solubilizing Microorganisms (PSM) like *Pseudomonas sp., Bacillus sp., Aspergillus sp. and Penicillium sp.* through various mechanisms of solubilization and mineralisation are able to convert inorganic and organic soil P respectively [9] into the bioavailable form facilitating uptake by plant roots. It is imperative to better understand the plant-soil-microbial P cycle with the aim of reducing reliance on chemical P fertilizers. This has led to increased interest in the harnessing of microorganisms to support P cycling in agroecosystems and for meeting agricultural challenges imposed by the still-growing demand for food especially in the semi-arid tropics. The main objective of the present study was to identify relationships between land use management practices and soil health in terms of availability of Phosphorus and PSM activity, relevant to a semi-arid zone as this, which has to face various atrocities of nature in terms of harsh climate, high salinity levels and drought.

2 MATERIALS AND METHODS

2.1 Study Site

The samples were collected from the agricultural fields in and around Bhuj, Kachchh, Western India (23º13'48.00''N, 69º42'35.06''E). According to NBSS and LUP, (National Bureau of Soil Survey and Land Use Planning, 2005) [10] the soils in this area belong to great group typic camborthids. The soils are sandy loams (calcareous). The agriculture of the area depends mainly on rains for irrigation but in some parts underground bore holes are used for irrigation. The average annual rainfall is 353 mm [11] with a coefficient of variation around 52%. Total number of rainy days varies between 12 and 20. June to September represent rainy months. May is the hottest month while January is the coldest month. Kachchh experiences extreme climatic conditions. This semi-arid zone of extreme part of western India is unique in various respects, due to its oppressive weather, low rainfall, aridity, hostile terrain and seismic instability. Over the years several natural and anthropogenic factors have affected the natural resource status of Kachchh. Drought and high salinity levels have remained a typical characteristic of the region. Owing to its unique ecological and geomorphological setting Kachchh is classified as biogeographic zone '3A' experiencing tropical arid climate [12].

2.2 Field Selection and Sampling Strategy

Different maize fields were classified based on the agricultural amendments applied viz: organic and integrated. Only those fields were chosen which had a past history of at least six years of applying the same amendment. The organic amendment (A1) included farm yard manure (FYM) applied before sowing at the rate of 4 ton/Ha, as a basal dose. In these fields, after sowing, a five day fermented concoction of cow dung, jaggery, soil, cow urine and gram flour was applied with watering twice, at seven days interval from sowing. In the integrated amendment fields (A2) FYM (farm yard manure) at the rate of 1 ton/Ha was applied as a basal dose before sowing. DAP (di ammonium phosphate) (40 Kg/Ha) + urea (60 Kg/Ha) was applied at 15-20 days after sowing. The sites selected for study comprised 8 fields on two farms (4 fields for A1 and 4 fields for A2). Two sampling seasons were considered Feb-May 2012 (season 1) and July-Oct 2012 (season 2). Soil Sampling was done at three phases of crop growth i.e. initial, fruiting and maturity stage (15, 45, 60 days after sowing {DAS}). In case of integrated fields A2 the first phase sampling (15 DAS) was done before the application of urea and DAP. Samples were collected from the rhizosphere of the maize crop upto the depth of 10-12 cm using standard soil sampling procedure. Four samples were collected per hectare and pooled to form one composite sample. Samples were analyzed in triplicates. Soil samples were divided into two parts, one part of the sample was air dried and sieved through a 2 mm sieve and analyzed for chemical characteristics and another part of soil sample was stored at 4°C for microbiological analysis.

2.3 Soil Chemical Parameters

Soil samples were analyzed for soil texture, according to the standard protocols [13]. The samples were analysed for pH and EC in a soil suspension of ratio 1:2 (w/v) with a glass electrode and digital EC meter respectively [14]; available Nitrogen by alkaline permanganate method [15]; available Potassium by flame photometer [14]; available Phosphorus following the Olsen's [16] method. The total P in soil samples was extracted by a mixture of concentrated sulphuric acid, hydrofluoric acid and hydrogen peroxide [17].

2.4 Soil Microbial Analysis

Microbial isolation was carried out from each soil sample after homogenously suspending in sterile saline solution (0.85% NaCl). After serial dilution soil samples were spread plate on PVK agar medium [18] and nutrient agar medium for isolation of PSM and total microbes respectively. Incubation was done at 37°C for three days. Transparent zone of clearing around colonies indicate P solubilization by microorganisms on PVK media. Persistence of their P solubilization capacity was checked in three subcultures in the same media. Microbial population was estimated by plate count method and reported as colony forming unit/gm soil.

2.5 Statistical Analysis

Results were expressed as MEAN ± S.E.M. To find out statistical significance between two amendments, students't test using the SPSS 17 software was performed. Level of significance was measured at $P \le 0.05$, 0.01 and 0.001.

3. RESULTS AND DISCUSSION

The available P status was higher in amendment 1 (A1) than amendment 2 (A2) ($P \le 0.01$, 0.001 and 0.05 respectively in all the three phases of crop growth viz. 15, 45, 60 days after sowing, as shown in Table 1. At 45 DAS in both seasons the fields with organic amendment (A1) recorded significantly higher available phosphorus ($P \le 0.001$) than the integrated amendment fields (A2) which also coincided with the higher population of PSM in A1 Figs. 1 and 2. Average of total microbial count, for three phases in A1 (3.06×10^9) (cfu/gm soil ×1000) was higher than A2 (7.15×10^3) (cfu/gm soil ×1000). Same holds true for season 2. However a comparison of the total Phosphorus levels in both the seasons was non-significant ($P \le 0.05$). It is clearly indicated that the soils in this semiarid zone of India are quite high in total P and a timely activity of PSM at this stage can make the native and applied P available. In integrated amendment a phosphatic fertilizer (DAP @ 40 Kg/Ha) was applied, still the P availability was significantly low, which points to the idea that even a good amount of P (native or applied) is of no use to the crops until and unless it is converted to a Soluble form through the action of P solubilizing microbes, which in turn can flourish well in good organic amendments.

Table 1. Effect of amendment 1 and amendment 2 on soil chemical and nutrient characteristics for season 1 and 2

DAS	Amendment	Ph	EC (dS/m)	Available Nitrogen (Kg/Ha)	Available Potassium (Kg/Ha)	Available Phosphorus (Kg/Ha)	Total Phosphorus (Kg/Ha)
				Season1			
15	A1	7.30±0.26	0.42±0.03***	406.27±1.89	255.062±5.68**	25.080±0.91**	106.945±9.84
	A2	7.82±0.18	0.75±0.04	370.15±49.40	158.370±14.06	10.685±1.12	106.112±10.81
45	A1	7.44±0.33*	0.50±0.04***	372.155±31.54	248.057±3.54**	20.587±1.04***	95.760±5.95
	A2	8.37±0.18	0.79±0.04	318.417±49.68	149.082±13.96	10.617±0.99	91.420±7.45
60	A1	7.53±0.20*	0.58±0.03**	306.552±37.10	199.502±11.55**	17.502±1.41*	87.192±4.99
	A2	8.64±0.23	0.89±0.01	282.670±32.34	119.597±10.07	9.915±0.34	78.435±8.61
				Season 2			
15	A1	7.20±0.34	0.36±0.02***	329.54±40.47	204.12±9.37**	26.04±2.12**	89.48±8.61
	A2	8.04±0.69	0.77±0.03	347.05±16.220	113.36±10.15	10.632±1.01	88.60±9.61
45	A1	7.46±0.30	0.45±0.04**	287.99±30.65	182.92±8.40**	20.207±0.97***	80.01±6.33
	A2	8.27±0.09	0.78±0.026	305.122±30.06	89.76±3.80	8.747±0.85	76.68±6.46
60	A1	7.46±0.35*	0.48±0.04**	272.52±23.21	171.12±9.42**	14.97±1.71*	74.59±4.72
	A2	8.87±0.11	0.83±0.02	252.00±17.29	87.57±3.77	8.31±0.3	71.46±8.23

*(DAS= days after sowing; Values are MEAN ± S.E.M., n = 4; *, **, *** represent significance at P ≤ 0.05, 0.01, 0.001 respectively as compared to A2; values without a superscript are non significant.*

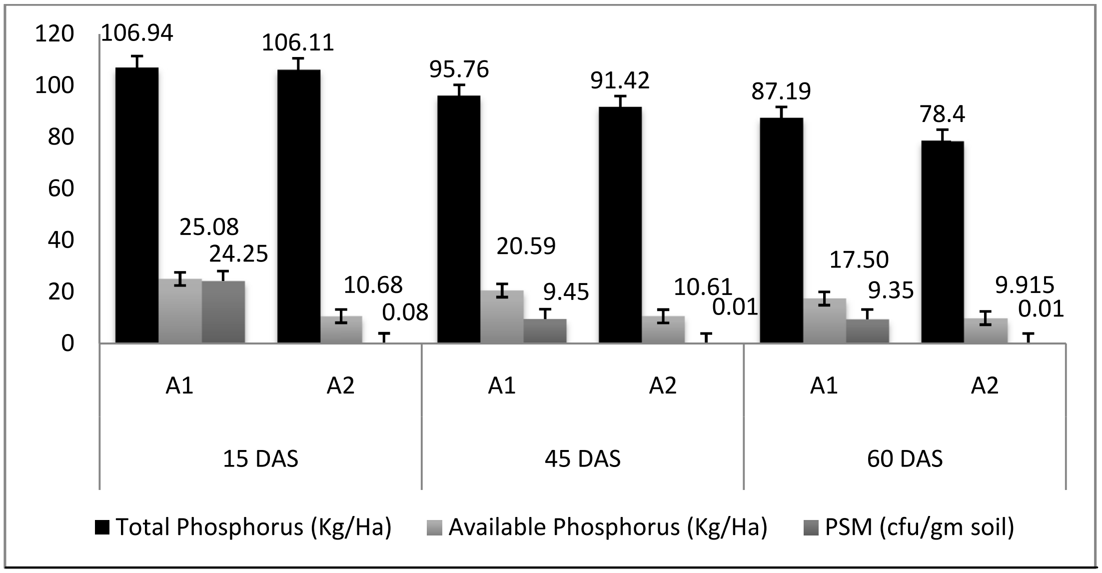

Fig. 1. Season 1 data for total Phosphorus, available Phosphorus and PSM count

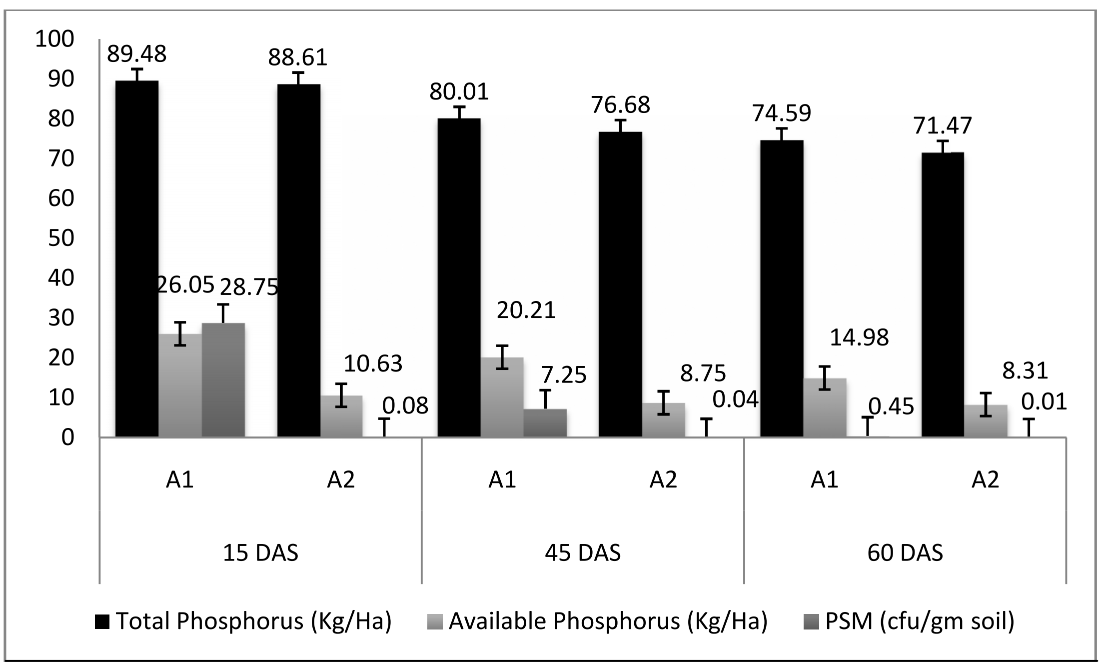

Fig. 2. Season 2 data for total Phosphorus, available Phosphorus and PSM count

The availability of P to plants is determined to no small degree by the ionic form of this element. The ionic form is determined by the pH of the soil in which the ion is found. By holding the pH of soils between 6.0 and 7.0 the P fixation can be kept at minimum [19]. The results show that the average pH value for A1 was around 7.3 while for A2 was around 8.3. The lower pH in organic amendments can be attributed to organic acids and humic substances released by organic manures which lower the pH to a range best suitable for P availability. These points to the idea that organic amendments not only increase microbial activity [20,21] but also help in maintaining soil pH at levels which help in P solubilzation. In A1 over time the pH increase is not that profound as it is in A2 and this can be attributed to the alkaline nature of chemical fertilisers which tend to increase soil pH in A2 over a period of time.

The range of electrical conductivity (EC) in integrated amendment (0.82 dS/m in season 1) was higher than organic amendment (0.65dS/m in season 1). It follows for the second season as well. Inorganic chemical fertilizers tend to increase the salinity of soil especially in areas as this, where increasing salinity is already a big concern [22,23]. Such increases in salinity due to input of chemical amendments in soils can pose a threat to the sustainability of the soil system over a long period of time. Singh et al. [24] studied the effect of continuous application of farm yard manure and chemical fertilizers on soil properties and found a decrease in pH of the soil by about one unit from the initial value and related it to the decomposition and mineralization of organic matter. Similarly, Bajpai et al. [25] observed a slight decrease in pH and EC at later stage of decomposition with application of organic manures. The available Potassium levels were higher in A1 than A2 (Table 1), this shows that the organic amendments were able to maintain a good nutrient system than integrated practices [26]. The fields analysed were practicing the same amendment practice since last 6 years so efficient soil management in farming systems can help to manage the good nutrient status of the soil in a sustainable manner. Various PS bacteria have also been isolated from stressed environments for example the halophilic bacteria *Kushneria sinocarni* isolated from the sediment of Daqiao saltern on the eastern coast of China, which may be useful in salt affected agricultural soils [27].

A comparison of available Nitrogen values were not statistically significant in both the seasons because the amendment A2 had externally applied N from urea fertiliser and in case of A1 it can be attributed to N fixing bacteria abundant in organic amendments.

4. CONCLUSION

Phosphorus is an important limiting factor in agricultural production, and considering the negative effects of chemical P fertilizers, microbial intervention of PSM seems to be an effective way to solve the phosphorus availability in soil. The study supports the fact that phosphorus could be immobilized at the peak of crop growth and if a good PSM count can be managed at this point then the unavailable Phosphorus can be made available to the crops. Management practices should, therefore, be geared towards making the release concur with peak crop nutrient demand. The study shows that organically amended fields had a higher available P status and the use of organic soil amendments has been associated with desirable soil properties as pH and electrical conductivity that can foster the working environment of beneficial microorganisms which in term help in maintaining a sustainable ecosystem in the long run. This fact holds true for semi-arid tropics where the scarcity of resources is a big issue and sustainability is a major concern, so organic based farming can deal with all these challenges.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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