1. INTRODUCTION

Phosphorus is an important nutrient in crop production. It promotes plant root growth and help in energy transformations as well as photosynthesis of plant. The function of phosphorus as a constituent of macromolecular structures is most prominent in nucleic acids, which, as units of the DNA molecule, are the carriers of genetic information and, as units of RNA, are the structures responsible for the translation of the genetic information. The major problem of phosphorus is its availability. The P cycle in soil is a dynamic system involving soils, plants and microorganisms. Major processes include uptake of soil P by plants, recycling through return of plant and animal residues, biological turnover through mineralization-immobilization, fixation reactions at clay and oxide surfaces, and solubilization of mineral phosphates through the activities of microorganisms. Chemical and biochemical aspects of the P cycle have been reviewed from several stand points, including fluxes of P on a global scale (Richey, 1983), pedogenesis (Walker, 1964), plant nutrition (Mengel and Kirkby, 1982) and inorganic forms and fixation reactions (Khasawneh et al., 1980), soil organic P and associated transformations (Anderson, 1980).

Phosphorus (P) is one of the major limiting factors for plant growth. The P transformations in soil involve complex processes like physical, chemical and biological. Plant availability of inorganic phosphorus (Pi) can be limited by formation of sparingly soluble Ca-phosphate, particularly in alkaline and calcareous soil; by adsorption to Fe and Al-oxide surfaces in acid soils, and by formation of Fe/Al-P complexes with humic acids (Gerke, 1992). Organic forms of phosphorus constitute a large proportion of soil P (often range varies from 20-80% of total soil P (Dalal, 1978). Plant roots acquire P as Pi
from the soil solution. Thus, to contribute to plant P nutrition, soil Po must be
dephosphorylated by phosphatase or phytase, which may be of plant and microbial in
origin (Tarafdar and Jungk, 1987, Tarafdar et al.; 2002, Dave et al., 2008, Yadav and
Tarafdar 2010). A variety of mechanisms has been proposed to account for the increased
mobilization of soil phosphorus in plant rhizosphere. These mechanisms include the
exudation reducing substances (Gardner, 1982); the production of mucilage and sloughed
off-root cells that provide an energy substrate for microbial activity in the rhizosphere
which subsequently dissolve mineral and organic P (Banik and Dey, 1981).

There are many forms of P with varying stabilities, which may be added to soil
by plants and animals. The most resistant to decomposition is inositol phosphate, which
makes up more than half the organic phosphorus in majority of soils. Although, some
soils contains up to 83% of the total organic P as inositol phosphates (Islam and Ahmed,
1973). The inositol phosphates in soils included esters, myochiro-, seyllo- and neo-
inositol of which only the first is common plant constituents, and known as phytic acid
$\left[C_6H_6(OHPO_3)_6\right]$, the other isomers might be of animal or microbial origin. The
phospholipids may come from plants, animals or microbes and the nucleic acids are at
least partly of microbial in origin and partly from recently added organic materials, but
they are broken down quite rapidly, so there are smaller residual amounts. The recovery
of soil organic P in the three principal forms follows the order: inositol phosphates >
phospholipids > nucleic acids. The same type of organic P compounds is found in plants,
but the order is reversed. The somewhat higher abundance of inositol phosphates in soils,
may be due to their tendency to form insoluble complexes with polyvalent cations, such
as Fe$^{++}$ and Al$^{+++}$ in acid soils and Ca$^{++}$ in calcareous soils.
Enzymes are important components involved in the dynamics of soil nutrient cycling. Enzyme activity in the soil environment is considered to be a major factor contributing to overall soil microbial activity and more recently, to soil quality. The mineralization and immobilization of phosphorus brought about by the activities of microorganisms or plant rhizosphere are, therefore, important links in the, general cycle of phosphorus in nature. A number of soil enzymes were reported for their significance in cycling of important plant nutrients like phosphatases for P-cycling, arylsulfatase for sulphur cycling, etc.

Inorganic ions dissolved in soil solution are the immediate nutrient source of the plants. In the case of phosphorus, only a small part of the amount taken up by a crop is present in soil solution at any time. Therefore, plant P supply depends largely on the movement of P-bound to the solid soil phase to soil solution. Major fractions of solid soil P known to contribute to P nutrition of plants are; sorbed-P, Ca- P, mainly P compounds of apatitic nature and organic P. The acquisition of these P sources may depend on plant properties which can differ among species (Foehse et al.; 1988).

Characterization of soil Pi and Po pools is fundamental for improving and understanding of P cycling in plant-soil system. Information about the fractionation of P compounds is needed to develop strategies to improve the use efficiency of soil P resources, especially where Po has been identified as a major source of bioavailable phosphate (Tarafdar et al.; 2001, Tarafdar et al.; 2002). Therefore, the aim of this work is, to quantify the contribution of soil microorganisms, fertilizer and plant to different P fraction, P-influx and P availability under different crops in Alfisols and Inceptisols. Therefore, the research entitled “Phosphorus mobilization in different crops on Alfisols and Inceptisols” with the following objectives:
(i). Phosphorus fractionation in different crops on Alfisols and Inceptisols.

(ii). Evaluation of different P-status in the crops at definite intervals.

(iii). To study the role of microorganisms in the crops on Alfisols and Inceptisols.

(iv). To find out the contribution of the crops, microorganisms and fertilizer on the phosphorus mobilization.

(v). Find out the P-influx/inflow in the crops system.
2. REVIEW OF LITERATURE

This chapter aims to provide reviews of related literature on current issues in mobilization of soil phosphorus research with the objectives to explore the best way to mobilize the soil phosphorus. The soil is a fundamental component of ecosystem and act as seat of biological activity and is conceived as a living organism (Skujins, 1967). It provides all the mineral nutrients for plant growth. Among these mineral nutrients, phosphorus (P) is one of the essential elements. Most soils contain a large amount of total phosphorus either as an inorganic or as an organic fraction. However, only a small proportion of the total soil P is available to plants i.e. availability of soil P fractions are a major limiting factor for crop production.

It has been widely recognized that the efficiency of applied P increases if P application rates are tailored keeping in view inherent phosphorus status of the soil. Phosphorus application causes variable effects and its content is built up in soils through series of transformations taking place; also these changes in environment of major and micronutrients in soils bring about different response patterns. Research work pertinent to the present investigation has been reviewed in this chapter. The chapter has been subdivided into the following sub headings for the convenience.

2.1 Mobilization of phosphorus.
2.2 Fractionation of phosphorus.
2.3 P-mobilization with microbial status in soil and plant roots.
2.4 Contribution of the crops, microorganisms and fertilizer on the P-mobilization.
2.5 Studies of P-influx/inflow to plants.
2.1 MOBILIZATION OF PHOSPHORUS

Incorporation of cover crops into cropping systems may contribute to a more efficient utilization of soil and fertilizer P than less P-efficient crops through exudation of P-mobilizing compounds by the roots of P-efficient plant species. This hypothesis was tested. Fractionation of P in the cluster root rhizosphere-soil indicated that white lupin can mobilize P not only from the available and acid-soluble P, but also from the stable residual soil P-fractions. In pot experiments with an acid Lurisol derived from low loss in available P, growth of wheat was significantly improved when mixed with white lupin due to improved P-uptake. Both in mixed culture and in rotation wheat could benefit from the P-mobilization capacity of white lupin, supporting the above hypothesis (Mahmoud Kamh et al., 1999, Yadav et al., 2005).

The numbers of workers have made the experiments on the mobilization of P under different soil types and different crop species. (Jungk et al., 1993; Grierson and Adams. 2000; Strom et al., 2002; Tarafdar et al., 2005; Panwar et al., 2005). Soil phosphateses which cause hydrolytic cleavage of phosphate for plant nutrition are contributed by plant (Tarafdar, 2008, Yadav and Tarafdar, 2007, Yadav et al., 2009) and microorganisms (Tarafdar and Chhonkar, 1979). Two types of evidences indicated that soil organic phosphorus undergoes mineralization. The first is based on changes in soil as a result of long continued treatments.

2.2 FRACTIONATION OF PHOSPHORUS

Inorganic ions dissolved in soil solution are the immediate nutrient source of plants. In the case of phosphorus, only a small part of the amount taken up by a crop is present in soil solution at any time. Therefore, plant P supply depends largely on the
utilization of P bound to the solid phase of soil. Major fractions of solid soil P known to contribute to P nutrition of plants are Sorbed-P, Ca-P, mainly P compounds of apatitic nature and organic P (Foehse et al., 1988).

Breaking prairie soils and cultivation with low fertilizer inputs in a wheat-fallow system, is known to have depleted soil phosphorus (P) contents of many North American grassland soils, largely by losses from the organic (Po) fraction (Hass et al., 1961; Tiessen et al., 1982, Tarafdar et al., 2006). Examination of particle size fractions from cultivated and similar uncultivated barren land silt loam soils showed that inorganic P (Pi) contents of coarse fractions increased during the first few years of cultivation and that a shift of soil P composition towards Pi at the expense of Po occurred in all size fractions with continued cultivation (Tiessen and Stewart, 1983).

P mineralization and immobilization may be determined by the net changes with time in soil organic and inorganic P. Following laboratory incubation under soil moisture content and temperature conditions favoring biological activity (Hedley et al., 1982) separates soil organic and inorganic P fractions according to their bio-availabilities, and can be used to monitor small changes in soil P content that occur during short term incubation experiments.

The adsorbed P that is bound to either Fe or Al is difficult to desorb by a simple equilibration with water and, therefore, is not readily available to plants. A variable proportion of the P adsorbed by kaolinite, goethite or Al-oxide is isotopically exchangeable. Experiments have shown that plants could utilize a considerable proportion of adsorbed P from geothite-P, kaolinite-P and Al-oxide-P complexes. It is not known whether this was a result of root activities or microbial transformations.
Different phosphorus fractions of Rajasthan soils have been studied by number of workers (Sacheti and Saxena, 1973; Vijay et al.; 1972; Ram Deo and Rahul, 1970). Transformation of added P into inorganic P fractions is influenced by number of factors and this aspect has been studied by number of workers for various soils of India (Singh and Pati Ram, 1977; Debnath and Hajra, 1972; Ranjodh Singh and Rahman, 1976; Sharma and Verma, 1980; Singh and Hari Ram, 1976; Gupta and Kamal Nayan, 1972; Singh et al., 2007).

Most of the P in grassland soils is in organic form. Organic forms are generally resistant to mineralization. Studies of changes in phosphorus in Great Plains soils (Haas et al., 1961) showed that losses of P by crop removal over period of up to 40 years were more than accounted for by decrease in organic P content. Thus organic P in these soils was serving as a reserve, which was being gradually depleted. Seasonal variation in organic P levels indicated a more dynamic nature of organic phosphates that generally recognized. Recent studies of P cycling in soils have also indicated the importance of rates of mineralization of organic P in regulating the supply of plant available P. These studies have demonstrated the fractionation of soil organic P that will separate the more actively cycling forms from those that have turn over time in the hundreds of years.

The distribution pattern of discrete forms of phosphorus in soil types and groups has been studied by various workers. Chang and Jackson (1957) reported that a dark brown soil and gray brown podzolic soil contained 68.95 per cent of the inorganic phosphorus in the form of calcium phosphate. Calcium phosphate in slate alluvial schist and saline alluvial soils; and that calcium, iron and aluminum phosphates are present in about equal amounts in sand stone and shale alluvial soils.
2.3 P-MOBILIZATION WITH MICROBIAL STATUS IN SOIL AND PLANT ROOTS.

Phosphatases capable of dephosphorylating organic P compounds in soils were first detected over sixty-five year ago, leading to interest in potentialities of organic P compound as fertilizers (Spencer and Stewart, 1934). Helal and Sauerbeck (1984) found increased phosphatase activity in the vicinity of maize roots but no significant change in organic phosphorus. Tarafdar and Jungk (1987) using a technique that divides the soil next to the root into layers about 0.2 cm thick showed that phosphatase activity at the root surface was up to eight times greater than in soil further away. An appreciable decrease in organic P very close to the root surface was also found by Dinkelaker and Marschner (1992). Moderate portion of organic phosphorus can be made available to plants by using microorganisms of higher potential of phosphatase activity as well as genotypes produced good amount of phosphatase (Boero and Thien, 1979; Helal and Sauerbeck, 1984; Tarafdar and Claassen, 1988). Tarafdar (1989) confirmed that alkaline phosphatase is solely of microbial in origin whereas both plants as well as soil microorganisms can produce acid phosphatase.

The efficiency of phosphatase treated with sodium glycerophosphate, lecithin and phytin were investigated as well as root exudates was also examined towards the breakdown of different organic P compounds to test the efficiency of plant produced phosphatase (Tarafdar and Claassen, 1988). Some plant species release large amounts of acid phosphatases and phytase from roots under P stress (Li et al., 1997), allowing mobilization and utilization of organic P in soil (Grierson and Adams, 2000). Plants can utilize P from all the organic sources used for the study almost as efficiently as inorganic sources. Organic P enhances more alkaline phosphatase activity. Lecithin influences more fungi and phytin influences more bacterial population. The amount of organic P
hydrolyzed surpasses plant uptake by a factor of 20 (Tarafdar and Claassen, 1988, Tarafdar and Gharu, 2006). This suggests that the limiting factor for the utilization of organic P by plants growing in soil is the availability of hydrolysable organic P sources.

It has been frequently reported that the activity of acid phosphatase in plant tissues (Silberbush et al., 1981; Szabo-Nagy et al., 1987; Flasinski and Rogozinska 1988) and secretion (Tadano and Sakai, 1991; Li et al., 1997; Trull et al., 1997) increases under P-deficient conditions as an adaptive mechanisms and to obtain P from organic sources. In sugar beet the activity of exogenous root phosphatase increased by 4 to 20 times and the kinetic parameters of acid phosphatase, $V_{\text{max}}$ and $K_{\text{m}}$, indicated higher hydrolyzing efficiency. More recently, Yadav and Tarafdar (2001) indicated that the plant starts secreting acid phosphatase as soon as their roots emerged (24-96h), moreover, the activities of the secreted acid phosphatase increased with plant age and were at a maximum under P-deficient conditions. A linear relationship between acid phosphatase activity and release of inorganic P from different organic P compounds has been observed, but the conditions under which the maximum secretion of acid phosphatase takes place, to most efficiently exploit the organic P compounds present in the soil, are unknown. In aseptic nutrient solution, roots of intact plants have readily hydrolyzed low molecular organic compounds such as sodium phytase, lecithin and sodium glycerophosphate (Tarafdar and Claassen, 1988, Saini et al., 2005, Kumar et al., 2007, Dave et al., 2008, Yadav and Tarafdar, 2009, Verma et al., 2010, Yadav and Tarafdar, 2010). The release rate of inorganic P even exceeded the P influx into roots. These compounds differ from those mainly occurring in soil, but it has been shown (Findenegg and Nelemans, 1993; Asmar et al., 1995; Seeling and Jungk, 1996) that at least part of the heterogeneous pool of organic soil P can be utilized as a P source of plants. Model calculations indicated that about one-third of the total P taken up by a
field-grown crop may have been derived from the organic soil phosphorus (Jungk et al., 1993).

The rate of P mineralization depends on microbial activity (Tarafdar et al., 1988) and on the activity of free phosphatase (Dalal, 1978), which is controlled by the solution P concentration (McGill and Cole 1981, Yadav and Tarafdar, 2001). In contrast, Yun and Kaeppler (2001) demonstrated that acid phosphatase may not be a major mechanism for scavenging or acquiring P and changes in acid phosphatase may reflect a state of P stress in plants. Other factors such as root architecture, secretion of low molecular weight carboxylates and microbial interactions might explain the differences. Inhibition of growth under low-P conditions is relatively less in the root than for the shoot, resulting in an increased root mass ratio (Goldstein et al., 1988). Tarafdar (2001) was critically assessed the rate of organic phosphorus (P) hydrolysis by wheat plants in soil solutions. The results suggested that the acid phosphatase secreted by two-week-old wheat plants increased with increase in organic P concentration up to 75 μM in soil solution. The rate of organic P hydrolysis depending on organic P concentration in soil solution was constant with time.

Phosphatase activity in the rhizosphere or soil solution may originate from plant roots (Tarafdar and Jungk, 1987; Dinkelaker and Marschner, 1992; Hayes et al., 1999), from fungi such as Aspergillus (Tarafdar et al., 1988), mycorrhizas (Tarafdar, 1995) or from bacteria (Tarafdar and Chhonker, 1979). Microorganisms may produce both acid and alkaline phosphatase (Richardson and Hadodas, 1997) but plants can only secrete acid phosphatase (Tarafdar, 1989). Hayes et al. (2000) found a small use of phytin by sterile growing germinating plants, which were increased by Aspergillus (fungus) phosphatase. In contrast Li et al., (1997a) found that acid phosphatase in the rhizosphere
of lupins were mainly of plant origin when compared under sterile and unsterile conditions. Plants phosphatase are also examined by Duff et al. (1994), Ozawa et al. (1995); fungal phosphatases by Huss et al. (1996), Wyss et al. (1998); mycorrhizal phosphatases by Dodd et al. (1987). It is conceivable, that P-deficiency not only causes changes in phosphatase quantity and activity, but also changes in isoenzyme composition. It could be possible, that P deficient plants not only release unspecific acid phosphatase, but also phytase (Li et al., 1997b).

The importance of soil organic P as a source of plant available P depends on its rate of solubilization and the rate of inorganic P release. Several types of phosphatases, such as phytases, are able to increase the rate of the dephosphorylation (hydrolysis) of organic P. In soil, the hydrolysis of organic P is predominantly mediated by the activity of soil microorganisms, although plant roots also possess phosphatase and phytase activity (Tarafdar and Jungk, 1987; Li et al., 1997). Microbial acid phosphatase was found to be more efficient in hydrolysis of organic P compounds than plant sources (Tarafdar et al., 2001). The potential role of phytase in increasing the availability of P from phytase in soils remains to be established. The additions of phytase increased the P content of maize seedlings when supplied with phytate, and it was concluded that the utilization of phytate by plant was limited by low rates of hydrolysis (Findenegg and Nelemans, 1993).

Helal (1990) carried out a comparative study of P utilization from inositol hexaphosphate by various cultivars of Phaseolus vulgaris in relation to the activity and characteristics of their root phosphatase. The cultivars showed significant differences in their uptake of P from inositol hexaphosphate with a clear dependency on the phosphatase activity of their roots at pH 5.0. The results suggested that root phosphatase
activity is a significant factor of phosphorous use efficiency (PUE) under limited mineral P supply.

Antibus et al. (1992) were examined selected naturally occurring mycorrhizase for differences related to efficiency of organic phosphorus hydrolysis in forest soils. All the roots of different growth stages had phosphatase activities with different substrates like PNP, phytic acid, bis-PNP etc. and having potential to mineralize different organic P compounds. The P-uptake was linked the phosphatase activity.

Gupta et al. (1998) determined the acid and alkaline phosphatase and phytase activities in the bacteroid-free fractions of chickpea (Cicer arietinum L.) nodules at different growing stages. They revealed that generally, acid and alkaline phosphatase activities were declined at 55 DASs after sowing and ATP was the best substrate for both phosphatases. The efficiency of acid phosphatase for utilizing frucotose-1, 6-bisphosphate as a substrate increased with nodule development, whereas acid phytase activity was low in nodules at all developmental stages and alkaline phytase and phytic acid could not detected. They also hypothesized that the higher content of water-soluble organic phosphorus in mature nodules would be due to the low activities of phosphatase at maturity. Helal and Sauerbeck (1991) concluded that root phosphatase play a significant role in the availability of organic phosphates under limited mineral P-supply.

George et al. (2002) reported that phosphatase activity was greatly enhanced in the rhizosphere of all agroforestry species. Agroforestry species enhanced the activity of acid phosphatase, while maize enhanced the activity of alkaline phosphatase. This suggests that agroforestry species are actively increasing rhizosphere phosphatase activity either directly by secretion or indirectly by stimulation of microbial activity and/or depletion of Pi. Overall, agroforestry species enhanced phosphatase activity in their rhizosphere. Also organic anion exudation and acid phosphatase activity of tree
roots may increase mobilization of P in the rhizosphere, the extent of which depends on the species, the organic anions and pH. However, it is unlikely that the extent of P mobilization will benefit adjacent crop plants unless crop roots exert insufficient P-mobilization effects themselves, and grow in the rhizosphere of tree roots (Radersma and Grierson, 2004).

Krasilnikoff et al. (2003) assessed the influence of variation in the lengths of roots and root hairs and rhizosphere processes on the efficiency of soil phosphorus (P) uptake. The results revealed that cowpea genotypes differed significantly in roots length, root hairs, P uptake and growth. Most of the total P uptake in the soil volume exploited by roots and root hairs was observed from the pool of non-Olsen’s P. This indicates a considerable activity of root-induced rhizosphere processes.

Plant alters soil P from the slowly exchangeable Pi pool to the rapidly exchangeable Pi pools in most soils (Chen et al., 2002). The specific P mineralization rates were significantly greater for soil under radiata pine than ryegrass which is associated to greater root phosphatase activity. It indicates that the growth of radiata pine enhanced mineralization of soil organic P.

The nature of organic phosphorus compounds has not been fully established. Organic-P in the soil may originate from the organic residues added to soil and also through synthesis by microorganisms. The largest fraction of organic-P approximately 50% appears to be in the form of phytin and its derivatives (Pederson, 1953; Dalal, 1978). Tarafdar and Jungk (1987) found an appreciable decrease in organic P hydrolysed by phosphatase activity, very close to the root surface. Tarafdar and Claassen (1988) suggest that phosphatase activity is not limiting factor in the use of organic P but rather the availability of phosphatase hydrolysable P compounds:
The accumulation of organic matter on wetlands is caused mainly due to lack of the oxygen needed for degradative microbial activity. Aeration during drainage and cultivation therefore spurs microbial transformations of the organic residues towards humus, mineral matter, CO$_2$ and water. The degree of decomposition of any organic deposit indicates the environment of its information and has an influence on the type and length of agricultural use that may be made of the deposit, because decomposition entails many physical and chemical changes, various properties have been used to assess the degree of decompositions or humification (Verma et al., 2010). Since the degree of decomposition is essentially a measure of the extent and rate of biodegradation of the organic matter, we felt it may be worthwhile to compare the various means used for measuring the extent of decomposing with the actual relative biodegradability of some unamended organic residues, and that was the aim of this study.

The mechanism of mineralization has been investigated in recent years and the role of micro-organism in carrying out the hydrolysis or mineralization of organic phosphorus compounds in soils has been highly appreciated. Large variety of microorganisms found in soil and rhizosphere cause mineralization of organic phosphorus (Verma et al., 2009). A considerable amount of work has been carried out with respect to the decomposition of organic phosphates by soil microorganisms that increasing supply of phosphorus decreased the phosphates activity especially of fungi.

The development of techniques to measure the amount of phosphorus (P) and other nutrients released from soil biomass upon fumigating (lysing) microbial cells coupled with more accurate staining methods for measuring microbial biomass have provided the means of examining the dynamics of P cycling in soils (Cosgrove 1967). In an earlier investigation (Chauhan et al. 1979) of aspects of P cycling in a chernozemic
black soil, the rate of P movement between soils inorganic (Pi), organic (Po) and biomass (P) compartments was measured following regular additions of grass and cellulose.

Microbial biomass P was affected slightly by addition of organic residue and/or fertilizer P to a soil having high available P status (34 ug.g$^{-1}$ resin - extractable P). The addition of fertilizer P did not change the percentage added cellulose-C (47%) remaining in the soil after microorganisms incubation. The monthly additions of cellulose without fertilizer P depleted the labile Pi pool by more than 25% in microorganisms. This suggested that the continued addition of cellulose without P for a longer period of time would eventually have exhausted the reserve of labile Pi leaving the microbial population dependent on the rate of mineralization of Po forms.

2.4 CONTRIBUTION OF THE CROPS, MICROORGANISMS AND FERTILIZER ON THE P-MOBILIZATION.

Condron et al., (1985) demonstrated that organic P could be nearly quantitatively extracted by using a combination of sequential acid and alkali extractions and ultra filtration. Hawkes et al., (1984) observed that significant mineralization of orthophosphate diester P occurred during 20 years of continuous fallow cultivation, while there was little change in the amount of orthophosphate monoester P over the same period. Other results have shown that orthophosphate monoester P accounted for a large proportion of the total organic P that accumulates in temperate soils as result of long term annual application of soluble phosphatic fertilizers (Hawkes et al., 1984; Condron et al., 1985).
Jones et al. (1999) postulated some facts or fictions for phosphorus mobilization by root exudates in the rhizosphere. They reported that root exudates generally release in greater quantities from plants under P starvation. While the exudation of some of these compounds is caused simply by an increase in membrane thickness under P deficiency, and the compounds have no capacity to mobilize soil P (e.g. sugar), other components such as organic acids can potentially mobilize significant quantities of soil inorganic P. This release of organic acids under P deficiency however, does not occur for all plant species, but it is particularly prevalent in non-mycorrhizal plants such as rape (Brasica napus) and lupin (Lupinus albus). In contrast, with an inadequate P supply most plants have the capacity to release phosphatase, which appear to be involved in the mobilization of soil organic P.

Zhang et al., (1998) reported that the retention of P by soil constituents, including the roles of soil microorganisms, plants roots, mycorrhizas and earthworm in the enhancement of plant availability of soil P. The mobilization of soil P by soil biota mainly involved the solubilization of insoluble inorganic P by excretion of protons and organic acids, and hydrolysis of organic P by phosphatase. The processes of P mobilization were regulated by the equilibrium between soil P supply and plant P demand. It is suggested that with an increase in the soil biota population and soil activity, P supply could be in synchronization with plant P demand. Roots with root hairs of Pallas absorbed nearly two times more phosphorus than the root hairless barley mutant, (Gahoonia et al., 2000, Panwar and Vyas, 2000, Saini et al., 2001, Panwar et al., 2003, Meena et al., 2004). Most of available inorganic P in the root hair zone (0.8 mm) was depleted as indicated by the uniform P depletion profile near its roots. The acid phosphatase activity near the roots of Pallas was higher and mobilized more organic P in the rhizosphere than the mutant. It suggests a link between root hair formation and
rhizosphere acid phosphatase activity. Hence, root hairs are important for increasing plant P uptake of inorganic as well as mobilization of organic P in soils.

Hedley et al. (1994) studied the phosphorus use efficiency and the forms of soil phosphorus utilized by upland rice cultivars and reported that in both P-fertilized and unfertilized soils, the majority of P taken up was solubilized from a 0.1M NaOH-soluble pool by root-induced changes. In unfertilized soil, significant difference between cultivars in internal P efficiency as measured by shoot dry weight per unit total plant P and also root growth and P uptake were strongly correlated with seed P-content.

The activity of extra cellular enzymes, including phosphatase, associated with different plant species is a function of the morphological and physiological attributes of root type. While many studies have reported significant correlations among the activity of soil enzymes, the microbial biomass, and other soil properties (Frankenburger and Dick, 1983; Speir et al. 1978 Eivazi and Bayan, 1996). The significance of individual plant species for phosphatase activity at the ecosystem scale remains largely unexplored. The contribution of individual species to soil phosphatase activity will be considerable when they produce specialized structures such as cluster roots which are known for their capacity to produce exudates (Dinkelaker et al. 1995 Saini et al., 2005).

2.5. **STUDIES OF P-INFLUX/INFLOW TO PLANTS.**

Plant species differ in their phosphorous use efficiency, ie the P content in soil needed to reach their maximum yield. The differences in external P requirement can be attributed to either a lower internal P requirement for optimum growth or higher uptake efficiency of the plants (Foehse et al., 1988). Therefore, P uptake not only depends on the amount of available P in soil but also on plant properties. Foehse et al., (1988) reported that plants have developed different strategies for P uptake from soil, while
some species increases the size of their root system and others increases the uptake per unit of root. These differences can be attributed to differences in root morphology (Barber, 1984) or mycorrhizal association (Yost and Fox, 1979) as well as chemical changes of the root environment (Gahoonia; 1987).

In phosphorus use there are two ways in which different P efficiencies can arise: (1) the efficiency with which P is utilized to produce yield i.e. the amount of P needed to the plant to produce one unit of dry matter (Loneragan and Asher, 1967). This is often called internal P requirement and is the P concentration in plants to produce 80% of maximum yield; (2) the uptake efficiency of the plant, which is the ability of the root system of acquire P from soil and accumulate it in the shoots. This depends on the capability of roots to absorb P, the active lifetime of roots, and on the amount of root per unit of shoot. It is well known that plant genus, species or even genotypes of the same species may vary in their ability to take up nutrients from soil (Gahoonia et al., 1999). This has been related to a combination of factors including root size and distribution (including root hair length and root surface), root exudations (H\(^+\), HCO\(_3^-\), reducing agents, chelates, organic anions, and enzymes), mycorrhizal infection, and transpiration rate (Foehse et al., 1988).

The overall interaction of soil and plant properties (nutrient availability and acquisition by plants) determines the rate and the quantity of P transfer into plants (Jungk, 1996). The differences in external P requirement can be attributed to either a lower internal P requirement for optimum growth or higher uptake efficiency of the plants (Foehse et al., 1988). Therefore, P uptake not only depends on the amount of available P in soil but also on plant properties. Various morphological (Hutchings and De Kroon, 1994; Gahoonia et al., 1997), physiological (Caldwell et al., 1992) and biochemical (Marschner, 1995; Ström, 1997) traits have been indicated to clarify the differences between genotypes in relation to phosphorus use efficiency (Marschner,
Analysis of phosphorus absorption characteristics in several crops suggests that P uptake efficiency depends on such traits as root-length density and architecture, root-hair length, mycorrhizal infection, root induced changes of the rhizosphere through exudates as well as high values of $I_{\text{max}}$ and low values of $K_m$.

Foehse et al. (1988) reported that plants have developed different strategies for P uptake from soil, while some species increase the size of their root system and other increases the uptake per unit of root. They also elucidate that P efficiency was related to the uptake efficiency of the plant, which is determined, by both root-shoot ratio and absorption rate per unit of root (influx). Species of low efficiency had low influx rates and low root: shoot ratio, whereas species of medium to high efficiency had either high influx rates (rape and spinach) or high root: shoot ratios (ryegrass and wheat). These differences can be attributed to differences in root morphology (Barber, 1984) or mycorrhizal association (Yost and Fox, 1979) as well as changes of the root environment (Gahoonia, 1987). The uptake or release of nutrients by roots growing in the soil leads to concentration gradients forming in the soil; the zone so affected is termed the rhizosphere. The nature of these gradients depends on three factors: the rate of uptake/release; the mobility of the nutrient in soil; and the rate of conversion between available and unavailable forms. The interplay between these factors determines the amounts of mineral nutrients acquired by the plant (Darrah, 1993).

Among the large number of factors controlling phosphatase activity, the P concentration in soil solution (McGill and Cole, 1981), the humus content (Tarafdar et al., 1989), the organic P substrates (Tarafdar and Claassen, 1988; Helal, 1990) and the application of pesticides (Spier and Ross, 1978) might be the most important when comparing the effects of different farming systems. As phosphatase is very stable in the soil, it gives only a partial measure of the actual soil biological activity (Maire, 1983). Exo-enzymes represent a permanent activity potential of biological origin (Burns 1982,
Nannipieri et al. (1983), which is consequently higher in biologically cultivated soils. Perrot et al. (1990) reported only slight temporal fluctuation in phosphatase activity while other biological soil parameters showed important temporal fluctuations. Roots and mycorrhizal hyphae depleted the soil Pi but did not influence the concentration of Po inspite of increased phosphatases activity in soil influenced by roots (Joner et al., 1995). That phosphatases activity was attributed to higher root length densities whereas mycorrhizal hyphae showed no influence on soil phosphatase activity.

Numerous authors have been revealed that net P uptake per unit weight of a plant is determined by root length, uptake kinetics and the root length per unit weight of the plant (Nielsen, 1993; Tinker and Nye, 2000). Low P-soil is characterized by a very low mobility of P, therefore, P has to be very close to the root cylinder in order to be bio-available (Barber, 1995; Tinker and Nye, 2000). Krasilnikoff et al. (2003) have been assessed the influence of variation in the lengths of roots and root hairs and rhizosphere processes on the efficiency of soil phosphorus (P) uptake. The results suggested that the genotypes of cowpea differed significantly in lengths of roots and root hairs, P uptake rates and growth. From 6 to 85% of total P uptake in the soil volume exploited by roots and root hairs was absorbed from the pools of non-Olsen-P, this indicates a considerable activity of root-induced rhizosphere processes.

Romer and Fahning (1998) reported that increase in P uptake of the Lolium multiflorum Lam. hybrids was related to the increase of root length. Thus the P uptake efficiency (influx per unit of root) remained unchanged between inbreeds and their hybrids. It is concluded that the heterosis effect corresponds to an increase of P uptake caused by an increase of the size of the root system. The activity of the root phosphatase increased with declining P status of shoots.

Chemical methods for determination of soil P available to plants do not take into account the effect of roots on P uptake by the plant. Moreover, the relative significance
of root parameters, as compared to soil supply parameters in determining P uptake, is unknown. In a critical analysis of the parameters involved in P uptake, Silberbush and Barber (1983) concluded that root growth rate and root radius were the most sensitive parameters influencing P uptake. Soil P supply parameters were more sensitive than root physiological uptake parameters. Phosphorus concentration in soil solution affected P uptake more than the diffusion coefficient and buffer power. Reduction of root radius while root volume was maintained constant by increasing root length increased P uptake. Where both soil volume and root volume were kept constant, reduction of root radius to the size of root hairs or mycorrhizal hyphae gave the greatest P uptake. Grain yield was correlated with P uptake per plant (Romer and Schenk, 1998). At tillering, the cultivars showed a significant variability in shoot biomass, P concentration, P removal, P influx, activity of acid phosphatase and root length.

Amijee et al. (1991) discussed P uptake models and utilization by plants in relation to some aspects of the chemistry of P in the soil and its transport to and absorption by the roots. The existing models include uptake by root hairs, local pH changes in the rhizosphere, effect of root exudates and VAM associations.

Among the large number of factors controlling phosphatase activity, the P concentration in soil solution (Mc Gill and Cole, 1981), the humus content (Tarafdar et al., 1989), the organic P substrates (Tarafdar and Claassen, 1988, Helal, 1990), and the application of pesticides (Speri and Ross, 1978) might be the most important when comparing the effects of different farming systems. As phosphatase is very stable in the soil, it gives only a partial measure of the actual biological soil activity (Maire, 1983). Exo-enzymes represent a permanent activity potential of biological origin (Burns 1982, Nannipieri et al., 1983) which is consequently higher in biologically cultivated soils. Perrot et al., (1990) reported only slight temporal fluctuations in phosphatase activity while other biological soil parameters showed important temporal fluctuations.
3. MATERIALS AND METHODS

The materials used and methods followed during the course of investigation are described as follows:

The present investigation was undertaken with two soil types belongs to soil order Alfisols and Inceptisols. The soils of these two orders dominate the major parts of Rajasthan. Further to find out the contribution of plants, dominated crops of the state were selected. The four crops viz. Maize, Sorghum, Wheat and Mustard were used in order to evaluate the plant, microbes and phosphorous fertilization effects on the phosphorous mobilization.

The rhizosphere and non-rhizosphere soil samples from the existing fields having all the four crops under study representing both the soil types were collected. These samples were analyzed for P fractions and mobilization at different crop growth stages for précised interpretations of pot experiment results.

**Materials used are as follows:**

### 3.1 SOILS

Soil orders selected for investigation represents the major part of soils in Rajasthan as per records of National Bureau of Soil Survey and Land Use Planning (Shyampura and Sehghal, 1995). Two soil orders namely Alfisols and Inceptisols were selected for experimentation in pots. The brief description of investigated soil orders is as mentioned below
3.1.1 Alfisols

The dominant property of this order soils is a coarse texture surface horizon overlaying a horizon with clay augmentation. These soils found in some parts of eastern and some parts of southern Rajasthan. Alfisols may have fragipans, duripans horizons, petrocalcic horizons or other feature that are used to define the various great groups. Most of the area covered by Alfisols of the Rajasthan falls under sub-order ustalfs and great group haplustalfs.

3.1.2 Inceptisols

Soils of this order shows minimal expression of development due to low intensity of accumulation of material, alteration of minerals, or other processes. Weak surface structure development or evidence of alteration and hydrolysis to release iron resulted in some reddening of soils. Inceptisols occupy a major portion in Rajasthan along the foothills of Aravallis and Vindhyan ranges. The most common sub-groups of ustochrepts found are typic, lithic, vertic and fluventic ustochrepts.

Table 3.1 Initial chemical characteristics of experimental soils

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Value of Udaipur</th>
<th>Value of Jhalawar</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Physical characteristics</td>
<td>Alfisols   Inceptisols</td>
<td>Alfisols Inceptisols</td>
</tr>
<tr>
<td>1</td>
<td>Mechanical analysis</td>
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<td></td>
</tr>
<tr>
<td>I</td>
<td>Sand (%)</td>
<td>53.30</td>
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<tr>
<td>ii</td>
<td>Silt (%)</td>
<td>20.70</td>
<td>21.50</td>
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<tr>
<td>iii</td>
<td>Clay (%)</td>
<td>26.00</td>
<td>29.70</td>
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<td>iv</td>
<td>Textural class</td>
<td>Sandy clay loam</td>
<td>Clay loam</td>
</tr>
<tr>
<td>v</td>
<td>Soil moisture (%)</td>
<td>28.50</td>
<td>36.45</td>
</tr>
<tr>
<td>S.No.</td>
<td>Parameters</td>
<td>Value of Udaipur</td>
<td>Value of Jhalawar</td>
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<td>-------</td>
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<td></td>
<td></td>
<td>Alfisols</td>
<td>Inceptisols</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td><strong>Chemical analysis</strong></td>
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<td></td>
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<td>i</td>
<td>Soil reaction (pH)</td>
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<td>ii</td>
<td>Electrical conductivity (dSm(^{-1}))</td>
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<tr>
<td>iii</td>
<td>Organic carbon (%)</td>
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<td>Cation exchange capacity (Cmol (P(^+))kg(^{-1}))</td>
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<td>Calcium carbonate (%)</td>
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<td>Available Phosphorous (kg ha(^{-1}))</td>
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<td>viii</td>
<td>Available Potassium (kg ha(^{-1}))</td>
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<td>Adsorbed -P (mg kg(^{-1}))</td>
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<td>Inorganic -P (mg kg(^{-1}))</td>
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<td>xii</td>
<td>Organic -P (mg kg(^{-1}))</td>
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<td>Calcium-P (mg kg(^{-1}))</td>
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<td>Aluminum-P (mg kg(^{-1}))</td>
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<td>Iron –P (mg kg(^{-1}))</td>
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<td>Occluded aluminum -P (mg kg(^{-1}))</td>
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<td>Occluded iron - P (mg kg(^{-1}))</td>
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<td><strong>Biological analysis</strong></td>
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<td>xxiii</td>
<td>Dehydrogenase (pKat g(^{-1}))</td>
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<td>1.688</td>
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<td>xxiv</td>
<td>Acid phosphatase (pKat g(^{-1}))</td>
<td>0.885</td>
<td>0.885</td>
</tr>
</tbody>
</table>
3.1.3. Fumigation of the soils

The experimental soil is divided in two halves. One half is used as such (non-fumigated) and another half is sealed under a polythene sheet and subjected to methyl bromide fumigation (200 kg methyl bromide containing; 10 g Kg\(^{-1}\) chloropicrin ha\(^{-1}\)). After four days the polythene sheet is removed and the soil is left for aeration.

The analysis of soil was done after 15 days of fumigation, to evaluate the effect of plant, microorganisms and phosphate fertilizer on the P-mobilization by using four crops (Maize, Sorghum, Wheat and Mustard).

3.2 FERTILIZER

Supply of phosphorous was given through Single Super Phosphate (16 % P\(_2\)O\(_5\)) granules. The whole amount of SSP as per experiment was applied to soil of pots and mixed thoroughly before sowing the crops. The recommended dose of phosphorous for Maize (40 kg P\(_2\)O\(_5\) ha\(^{-1}\)), Sorghum (40 kg P\(_2\)O\(_5\) ha\(^{-1}\)), Wheat (60 kg P\(_2\)O\(_5\) ha\(^{-1}\)) and Mustard (40 kg P\(_2\)O\(_5\) ha\(^{-1}\)) was applied to experimental crops.

3.3 CROPS

Crops selected for investigation are major growing crops of Rajasthan. Out of four crops selected for the study Maize and Sorghum are main staple food crops of *kharif* where as Wheat is a main cereal crop of *rabi* and Mustard as oilseed crop.

To confirm the research findings with applied aspects composite or high yielding cultivars of Maize (Navjot), Sorghum (CSV-10), Wheat (Raj.3077) and Mustard (Pusa Bold) were selected for study purpose.
3.4 EXPERIMENT DESIGN AND LAYOUT: THE DETAILS ARE AS FOLLOWS:

Crops: 4 (i) Maize (Var. : Navjot)

(ii) Sorghum (Var.: CSV-10)

(iii) Wheat (Var.: Raj-3077)

(iv) Mustard (Var.: Pusa Bold)

Treatments:

1. Soils: 2 (i) Alfisols (A₁) (ii) Inceptisols (A₂)

2. Fumigations: 5

(i) Fumigated soil without plant (Control) (B₁)

(ii) Fumigated soil with plant (Plant effect) (B₂)

(iii) Non-fumigated soil with plant (Plant + microorganism effect) (B₃)

(iv) Fumigated soil with plant and recommended dose of SSP (Plant + fertilizer effect) (B₄)

(v) Non-fumigated soil with plant and recommended dose of SSP (Plant + M.O. + Fertilizer effect) (B₅)

3. Treatment combinations: Soils x Fumigations: 2 x 5 = 10

4. Replications: 4

5. Total number of pots per crop = No. of replications x No. of treatment combinations 4 x 10 x 6 (six intervals) = 240
3.5 FILLING OF THE POTS WITH SOIL AND APPLICATION OF FERTILIZER DOSE

Earthen pots (30 X 20 cm size) were thoroughly cleaned with water. Bottom hole of the pot was covered with earthen piece followed by addition of acid washed quartz up to a height of 1.5 cm in order to provide proper drainage. For each soil groups, composite soil sample was prepared by taking samples. Soil samples were air dried in shadow, gently crushed with a wooden roller and passed through 2 mm sieve. Processed soil samples were used for fumigation, laboratory analysis and pot experiments. Ten kilogram of 2 mm sieved fumigated and non-fumigated soil was transferred separately in each pot as per treatment. Phosphorous was applied through SSP granules 17.8 mg per 10 kg soil for maize, sorghum and mustard, and 26.7 mg per 10 kg soil for wheat as a basal dose and mixed well in whole pot soil.

3.6 CROP RAISING AND IRRIGATION

Total 240 pots were taken for pot experiment in which three seeds of each crop were sown by wooden dibbler at equal distance. After emergence of seedlings, one seedling was maintained at definite intervals of crop growth (i.e. 15, 30, 45, 60 DAS and at harvest) separately in the pots in four replications. Pots were irrigated at 50 % moisture at field capacity. Layout direction of pots was regularly rotted to avoid the environmental effect due to the position of pots. The incidence and management of disease and pests was checked regularly throughout the growth period.

3.7 COLLECTION AND PREPARATION OF SOIL SAMPLES

Two separate bulks of surface soil (0-15 cm) belonging to Alfisols and Inceptisols respectively, were collected. Samples of rhizosphere and non- rhizosphere soil were also collected from the field of existing crops and analyzed for P-fractions at three stages (before sowing, 45 DAS and at crop harvest). Soil sample were drawn by mini soil augor up to depth of 20 cm of the pots at different four places at six intervals,
separately in the individual pots. Collected soil samples were mixed well, air dried and crushed by wooden roller and passed through 2 mm sieve. Processed soil samples (< 2 mm) were subjected to subsequent laboratory analysis.

3.8 HARVESTING AND PREPARATION OF PLANT SAMPLES

After the required crop growth period (15, 30, 45, 60, 75 DAS and at harvest) plants were harvested along with roots in order to avoid any contamination from the soil separately in the pots. The plant samples were dried in hot air oven at constant temperature (70°C) and dry weight was recorded after complete drying. Dried samples were grinded by mechanical grinder and subsequently analyzed.

3.9 POT EXPERIMENT STUDIES

3.9.1 Physical parameters of soil

3.9.1.1 Soil Moisture

The soil moisture was determined by gravimetric methods. A fresh soil sample was weighed and dried in an oven at 105°C. After drying to a constant weight, the soil was reweighed. The per cent moisture content was calculated by using the following formula:

\[
\text{Per cent moisture} = \frac{\text{Moist soil weight} - \text{Oven dry soil weight}}{\text{Oven dry soil weight}} \times 100
\]

3.9.1.2 Field Capacity

The field capacity was determined as described by Jalota et. al. (1998). The sieved soil was uniformly packed in the cylinder leaving the upper 10 cm of the cylinder unfilled. A glass tube was pushed in it to work as capillary tube to facilitate the exit of displaced air through it. Now, sufficient amount of water was added on soil surface in order to fully saturate soil column except the lower 10 cm. The soil surface was sealed
with paraffin. Cover the column with a watch glass to check evaporation and also plugged the protruding glass tube with cotton to reduce evaporation. The cylinder was allowed to stand for 48-72 hours. After redistribution of applied water, the soil column in 2 cm increments was sampled leaving 10-12 cm of the top soil and the moisture content of the samples is determined. It was calculated by following formula:

\[
\text{Percent water content} = \frac{\text{Wet soil weight} - \text{Oven dry soil weight}}{\text{Oven dry soil weight}} \times 100
\]

3.8.1.3 Bulk density

The bulk density of the soil is the mass of the soil per unit volume. A 50 ml capacity volumetric flask was taken and the bulk density was calculated in terms of per unit volume as described by Jalota et al. (1998).

3.9.1.4 Soil particle analysis

The mechanical analysis of the soil was determined by Bouyoucos hydrometer method (Bouyoucos, 1962). The method is based on the Stoke’s Law which states that the rate of fall of particles in a suspension is directly proportional to their size. Accordingly, larger particles (sand) will settle first followed by silt and then clay.

100 g soil sample was taken in a 500 ml beaker and organic matter content was destroyed by giving treatment of 30% H₂O₂. Added 200 ml distilled water, 100 ml of the sodium hexametaphosphate solution (a dispersing agent), stirred well and kept for over night. Transferred the contents quantitatively on the next day to the cup of the mechanical stirrer by giving 4-5 washings of distilled water. The volume was made upto 500 ml. The content was stirred vigorously and then transfer to the suspension cylinder (1000 ml), after giving 4-5 washing of distilled water the volume was made upto 1000
ml. The cylinder was kept on flat Table, the stopper was removed and immediately placed the hydrometer in the suspension. The reading was recorded exactly 40 seconds after placement of hydrometer. Repeated the same procedure for 2 hr reading. A blank was run simultaneously without soil and the room temperature was recorded in °F. The calculation was done as described below:

Correction factor (CF) = (Actual room temperature in °F – 68) × 0.2

\[
\text{Per cent silt + clay} = \frac{(S-B) + CF}{\text{Weight of sample (g)}} \times 100
\]

Where, S and B stand for sample and blank readings, respectively, taken at 40 second (first reading)

\[
\text{Per cent clay} = \frac{(S-B) + CF}{\text{Weight of sample (g)}} \times 100
\]

Where, S and B stand for sample and blank readings, respectively taken after 2h.

Percent sand = 100 – (silt + clay)

3.9.2 Chemical Analysis

3.9.2.1 Soil Reaction (pH)

The pH of soil samples was determined by soil water suspension ratio (1:2.5). Twenty gram of each soil samples were weighed and 50 ml of distilled water was added to it. Thereafter, the samples were shaken in shaker for half an hour and pH of the soil was determined by using pH meter.
3.9.2.2 Electrical Conductivity

The electrical conductivity was measured by electrical conductivity meter in the same soil: water suspension ratio (1:2.5) as used for pH.

3.9.2.3 Organic Carbon

Organic carbon was determined by Walkley-Black method (1934). Five hundred mg of air dried and sieved soil was taken in a 500 ml conical flask. 10 ml of 1N K₂Cr₂O₇ solution and 20 ml of concentrated H₂SO₄ was added to it and mixed by gentle rotation for one minute, to ensure complete reaction of the reagent with the soil. This was allowed to stand for 30 minutes. A blank (without soil) was run in same way. After complete oxidation, 200 ml of distilled water, 10 ml of 85% ortho-phosphoric acid and 1 ml diphenylamine indicator was added to it. The solution was back titrated with 0.5 N ferrous ammonium sulphate solution delivered from a burette. The colour was dull green with chromate ion at the beginning, then changes to a turbid blue as the titration proceeds. At the end point, this colour sharply changes to a brilliant green. Organic carbon was determined by using the following formula:

\[
\text{Organic carbon (\%) = } \frac{10 \times (B - S)}{B} \times 0.003 \times \frac{100}{\text{Weight of sample (g)}}
\]

Where, B and S stand for the titration value (ml) of blank and sample, respectively 1 ml of 1 N K₂Cr₂O₇ = 0.003 % carbon

3.9.2.4 Calcium carbonate (CaCO₃)

Determination of calcium carbonate was carried out as per procedure outlined by Piper (1950).
3.9.2.5 Cation exchange capacity (CEC)

It was determined by method proposed by Richards (1954) by saturating the soil with ammonium ions.

3.9.2.6 Available Phosphorus

To estimate available phosphorous 2.5 g of air dried sieved soil was taken into a plastic bottle. 50 ml of 0.5 M NaHCO₃ (pH 8.5) plus a tint of activated charcoal was added to it. This was shaken on a horizontal shaker for 30 minutes. Thereafter, the solution was filtered through a whatman no. 42 filter paper. 5 ml of aliquot was taken in 25 ml volumetric flask and two to three drops 2,4-dinitrophenyl indicator was added and then discolour the suspension using 0.5 N HCl or 6N NH₄OH (as per requirement) solution drop by drop. Five ml of ammonium molybdate solution was added to it and the colour was developed by adding 1 ml of 2.5% SnCl₂. The blue colour intensity was measured spectrophotometrically at 660 nm wavelength using red filter. The μg/g or μg/ml available phosphorus was calculated by using the formula:

\[
P (\mu g/g) = \frac{\text{Absorbance} \times \text{final vol.} \times \text{total aliquot}}{1 \text{ ppm standard reading} \times \text{initial vol.} \times \text{Weight of soil sample}}\]

3.9.3 Estimation of different phosphorus parameters:

3.9.4.1 Available phosphorus.

Dickman and Bray's (1940) method for colour development : An aliquot of the phosphorus containing test solution was pipette into a 50 ml volumetric flask, adjusted to pH 3 with 4N ammonium hydroxide or 4N hydrochloric acid using 2-4, dinitrophenol as indicator and the volume raised to 35 ml. 10.0 ml. of the chloromolybdic acid solution
(1.5%) were added followed by addition of 5 ml. of stannous chloride (10 grams stannous chloride dissolved in 25 ml. concentrated hydrochloric acid, one ml. from stock solution was diluted to 66 ml. with distilled water) solution and colour of the solution was measured in Klett Summersion Colorimeter at 660 nm Wave length.

3.9.4.2 Soil Phosphorus Fractionation

Fractionation of inorganic soil phosphorus was carried out according to the method of Chang and Jackson (1957).

3.9.4.2.1 Adsorbed Phosphorus (Adsorbed-P)

One gram soil sample was taken in 100 ml. centrifuge tube and extracted by shaking with 50 ml. of 1 N ammonium chloride for 30 minutes to remove adsorbed phosphorus and exchangeable calcium. The suspension was centrifuged and phosphorus was determined in supernatant liquid according to the method of Dickman and Bray (1940).

3.9.4.2.2 Aluminum Phosphate (Al-P)

Soil left after removal of adsorbed phosphorus was shaken with 50 ml. of neutral 0.5N ammonium fluoride for 1 hour. The suspension was centrifuged off. A suitable aliquot (5-10 ml) was taken for colour development. 15 ml. of 0.3 M boric acid were poured in each volumetric flask containing 5-10 ml aliquot. Colour was developed by Dickman and Bray’s method (1940) and comparison of colour was done at 660 nm wave length.

3.9.4.2.3 Iron Phosphate (Fe-P)

Soil sample saved after determination of aluminum phosphate was washed two times with 25 ml of saturated sodium chloride solution. The washed soil sample was
shaken with 50 ml of 0.1N sodium hydroxide for 17 hours. The suspension was centrifuge off for 15 minutes at 2400 rpm to obtain clear supernatant solution. Re-centrifugation was done whenever necessary. The solution was decanted in another centrifuge tube and soil sample was saved for calcium phosphate determination. Two ml of 2 N sulphuric acid was added to the decanted solution and few drops of concentrated sulphuric acid were added until the organic colloids began to flocculate. The suspension was centrifuged off. An aliquot was adjusted to pH 3 by using para-nitrophenol indicator and yellow colour was removed with the help of 2N hydrochloric acid added drop wise. Final colour development was carried out according to the Dickman and Bray's (1940) method.

3.9.4.2.4 Calcium Phosphate (Ca-P)

 Soil samples saved after iron phosphate determination were washed twice with 25 ml. of saturated sodium chloride solution, followed by extraction with 50 ml of 0.5N sulphuric acid by shaking for one hour. The suspension was centrifuged and the supernatant liquid was decanted into clean flask. The soil sample was saved for subsequent occluded iron phosphate determination. An aliquot (1-5 ml) from supernatant solution was taken for estimating calcium phosphate. The aliquot was adjusted to pH 3 using para-nitrophenol. Final colour was developed according to Dickman and Bray's (1940) method.

3.9.4.2.5 Occluded iron Phosphate (occluded Fe-P)

 Soil sample after removal of calcium phosphate was washed twice with 25 ml of saturated sodium chloride solution. The soil was then suspended in 40 ml of 0.3M sodium citrate solution and 5 ml of 1N sodium bicarbonate solution were added. Chang and Jackson's (1957) method was followed subsequently with slight modification.
3.9.4.2.6 Occluded aluminum phosphate (occluded Al-P)

After removal of occluded iron phosphate the soil sample was washed twice with 25 ml of saturated sodium chloride solution. Further extraction was carried on as described under aluminum determination.

3.9.4.2.7 Estimation of organic and inorganic Phosphorus

Organic phosphorus was determined by extraction method as described by Mehta et al. (1954). Inorganic phosphorus was determined in 10 ml aliquot, using gradient elution technique after removal of turbidity which appeared in the first three tubes using Jackson (1973) method.

3.9.4.4 Mineral phosphorus and organic phosphorus

**Extraction:** Mineral phosphorus and organic phosphorus of the soil were estimated by the procedure of Mehta et al. (1954) as describe by Jackson (1967) 1.0 g air-dried and sieved soil sample is placed in 100 ml centrifuge tube and 10 ml of concentrated HCl was added. The suspension was heated on a steam plate for 10 minute (final solution temperature about 70°C), and an additional 10 ml of concentrated HCl was added and mixed. This suspension was allowed to stand at room temperature for 1 hour. After addition of 50 ml of water the suspension was centrifuged and the clear supernatant liquid was poured into a 250 ml volumetric flask containing about 50 ml of water.

In the residual soil 30 ml of 0.5 N NaOH was added and after sterring the suspension was allowed to stand at room temperature for 1 hour. The suspension was then centrifuged and the supernatant liquid was poured into the volumetric flask containing the acid extract. Thereafter 60 ml of 0.5N NaOH was added in the tube and the tube was covered with an inverted 50 ml beaker, followed by warming in an oven at 90°C for 8 hours. The tube was cooled, the suspension centrifuged, and the supernatant
liquid was poured into 250 ml flask containing the previous extracts. The combined extracts were diluted to known volume with water and mixed thoroughly.

**Digestion:** 15 ml aliquot was pipetted out from combined extractant and 1 ml of 72 percent HClO₄, was added and placed on a steam plate. The temperature was raised until fumes of HClO₄ appear. The flask was covered with a watch glass to reduce further loss of the acid. The digestion was continued until the colour of the solution no longer changes. The flasks were then cooled and the contents were transferred quantitatively to a 50 ml volumetric flask with the aid of a rubber policeman to ensure complete transfer of the silica.

**Estimation:** 5 ml of aliquot of both extracted and digested suspensions were taken in a 25 ml volumetric flask and neutralized with 6N NH₄OH to yellow in presence of p-nitrophenol indicator and added with 0.5 N HCl just to colourless. Then 20 ml of water, followed by 5 ml of chloromolybdic acid was added with thorough mixing. Finally 3 drops of chlorostannous acid solution was added, followed by immediate mixing. A blank was carried simultaneously with distilled water. The percentage transmittance was read at 660 nm after 10 minutes, using red filter. The total and mineral phosphorus extracted were calculated as ppm of P in the soil. The organic phosphorus was calculated as follows:

\[
\text{ppm of organic P} = \text{ppm of total P} - \text{ppm of total inorganic P (mineral P)}
\]

3.9.5 Biochemical Analysis

3.9.5.1 Dehydrogenase

Monitoring of dehydrogenase, which are respiratory enzymes and integral part of all soil organisms, will give a measure of biological activity of soil at a given time. In respiration biological oxidation of reduced compounds occur which is catalyzed by
dehydrogenase. During this process energy is evolved. The process can be represented as:

\[ RH_2 + A \rightarrow R + AH_2 (2H^+ .2e^-) \]

Where \( RH_2 \) represents a reduced compound (hydrogen donor) and ‘\( A \)’ is the electron acceptor which in aerobic organisms is oxygen. Under anaerobic conditions, compounds like 2,3,5 triphenyl tetrazolium chloride (TTC) can act as electron acceptor. In the process TTC gets reduced to a pink coloured compound Triphenyl formazan (TPF), which can be quantitatively extracted by methods and measured spectrophotometrically

\[ TTC + 2H^+ .2e^- \rightarrow TPF \]

The dehydrogenase can be assayed as rate of formation of TPF from TTC. Higher the biological activity faster will be the formation of TPF.

Dehydrogenase assay were measured in soils immediately after soil sampling or in stored soils at 4°C (within 15 DASs). Dehydrogenase activity was assayed by the method of Tabatabai (1982). One gram of soil sample was taken in a screw cap test tube (15 ml capacity). To it, 0.2 ml of 3% TTC and 0.5 ml of 1% glucose was added. After mixing the content, the tubes were incubated for 24 hours at 30°C. Once the process of incubation was over, it was followed by addition of 10 ml of methanol to it. The whole material was mixed thoroughly for 1 minute. After mixing, the tubes were placed in refrigerator for 3 hours. The production of Triphenyl formazan was determined by measuring absorbance at 485 nm.

3.9.5.2 Acid phosphatase and alkaline phosphatase

The acid and alkaline phosphatase was analyzed by adopting the standard procedure of Tabatabai and Bremner (1969). The procedure described for assay of
phosphomonoesterase activities are based on colorimetric estimation of the para-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 5.4 for acid phosphatase and pH 9.2 for alkaline phosphatase) sodium para-nitrophenyl phosphate solution.

Soil sample (1.0 g < 2 mm) weighed in 15 ml capacity screw cap test tube. Added 0.2 ml of toluene, 4 ml of para-nitrophenyl phosphate solution prepared in acetate (pH 5.4 for assay acid phosphatase) and borax- NaOH buffer (pH 9.2 for assay of alkaline phosphatase) was added and swirl the test tube for a few seconds to mix the contents. The test tube was covered with screw cap, and placed it in an incubator at 35°C. After 1 hour, the cap was removed and, 1 ml of 0.5 M CaCl$_2$ and 4 ml of 0.5 M NaOH was added. Swirl the test tube for a few seconds, and filter the soil suspension through a whatman no. 42 filter paper. The yellow colour intensity of the filtrate was measured spectrophotometrically at 420 nm wave length. The para-nitrophenol content of the filtrate was calculated against the standard curve obtained with standards containing 0, 10, 20, 30, 40 and 50 μg of para-nitrophenol.

3.9.5.3 Phytase activity

Phytase activity was assayed by the method of Ames (1966). 2.0 g sieved soil (2 ml aliquot incase of solution culture experiments) was placed in 15 ml capacity screw cap test tubes. 4 ml of 100 M sodium acetate buffer (pH 4.5), and 1 ml of sodium phytate (1μM) was added and incubated at 37°C for 1 hour. After 1 h incubation, the reaction was terminated by the addition of 0.5 ml 10% Trichloro acetic acid (TCA) (CCl$_3$COOH). Proteins precipitated by TCA were removed by centrifugation at 10,000 g for 10 minute and the supernatant was analyzed for liberated inorganic P, using chlorostannous reduced
molybdophosphoric hydrochloric acid method as described by Jackson (1957). One unit of phytase activity was defined as the amount of enzyme, which liberated 1 \( \mu \) mole Pi per minute.

3.10 PLANT ANALYSIS

3.10.1. Dry matter yield

Oven dried weight of the plant samples was recorded.

3.10.2. Uptake of phosphorus

The uptake of phosphorus contents was calculated by using the data per cent phosphorus and dry matter yield. The formula for calculation is as under:

\[
P \text{ Uptake (mg pot}^{-1}\text{)} = \text{Dry matter yield (g pot}^{-1}\text{)} \times \text{Phosphorus content (per cent)} \times 10
\]

3.10.3. Per cent phosphorus recovery

The per cent phosphorus recovery calculated by formula is as given below:

Per cent recovery of P = \([T-C/a] \times 100\]

Where, \( T \) = is uptake of phosphorus in treated plot,

\( C \) = is uptake in control plot

\( a \) = is phosphorus supplied.

3.10.4. Phosphorus use efficiency (PUE):

\[
PUE = Y_{dt} - Y_{dc}/Pa
\]

Where,

\( PUE \) = Phosphorus use efficiency (g straw produced of P\(_2\)O\(_5\) applied)
Ydt = Yield in treated pot (g pot$^{-1}$)

Ydc = Yield in P control pot (g pot$^{-1}$)

Pa = Phosphorus applied (g pot$^{-1}$)

3.10.5 Per cent yield response:

Per cent yield response calculated by formula:

\[
\text{Per cent yield response} = \frac{\text{Yield at the nutrient level} - \text{Yield of the control (no nutrient)}}{\text{Yield at the nutrient level}} \times 100
\]

3.10.5 Analysis for P-content

Grinded and sieved (< 2 mm) plant samples were digested, using tri-acid mixture as described by Jackson (1957). Placed 0.5 g plant sample in 100 ml cooled conical flask and then added 5 ml of tri-acid mixture (HNO$_3$: H$_2$SO$_4$: HClO$_4$, 9:2:1). The digestion was carried out at 180 to 200 °C until a clear solution remains after the acids were largely volatilized. After complete digestion the final volume was made up to 50 ml.

For phosphorus estimation 5-10 ml (5 ml for low P-content and 10 ml for high P content plant samples) of digested plant material was placed in 50 ml volumetric flask, 20 ml distilled water and 15 ml mixture of dilute HNO$_3$, Ammonium molybdate (5%) and Ammonium metavanadate (0.25%) in equal proportions (1:1:1) was added to it. The volume was made upto 50 ml with distilled water and read the yellow colour intensity at 485 nm using spectrophotometer as described by Kitson and Mellon (1944). The P-content was calculated as $\mu$g P g$^{-1}$ plant material.
### 3.10.6 Root Length

Root length was measured, using the line intercept method based on Tennant (1975). Principle of this method is that root length can be estimated by counting the number of intersections between roots and sample lines. In this method roots were spread out with random orientation in a thin layer of water on a glass plate (about $25 \times 25$ cm) marked in grid lines. All intersections of roots with gird lines (taking the upper or left boundary of the line as criterion in case of doubt) are counted (Fig. 3.1). Results for horizontal (H) and vertical (V) grid lines are added to number N. If the grid size was D (mm), root length L (mm), then the equation stands:

$$L = \frac{\pi ND}{4}$$

![Figure 3.1: Line intercept method for determining root length by counting the number of interceptions between roots and horizontal (H) plus vertical (V) lines of a grid](image)

Fig. 3.1: Line intercept method for determining root length by counting the number of interceptions between roots and horizontal (H) plus vertical (V) lines of a grid
3.10.7 Computation of Plant Growth Parameters

The root weight, shoot weight and root: shoot ratios were calculated on dry weight basis. The P inflow/influx was expressed per unit root length (cm) and time (second) between the two consequent harvestings/samplings. The P-influx was calculated using the formula after Romer and Schenk (1998) as follows:

\[
P\text{- Influx} = \frac{u_2 - u_1}{L_2 - L_1} \times \frac{L_2}{t_2 - t_1}
\]

Where \(u_2-u_1\) (\(\Delta U\)) is the change in P-uptake (mol plant\(^{-1}\)), \(L_2-L_1\) (\(\Delta L\)) is the change in total root length (cm plant\(^{-1}\)) at \(t_1\) and \(t_2\) and \(t_2 - t_1\) (\(\Delta t\)) the change in time (second). The P-influx was expressed as (millimole P/Pot/Sec X 10\(^{-11}\)).

3.11 Statistical Analysis and Interpretation:

All the data of different observations were stastically processed in order to give proper interpretation to the difference obtained due to influence of different treatments. Analysis of variance was carried out by the method described by Fisher (1949) and Panse and Sukhatme (1967). The comparisons among means were made by calculating by critical difference, whenever they were found significant through F-test at 1 and 5 per cent level of significance.

The laboratory data were properly interpreted in order to draw appropriate conclusions with special reference to variability, various relationship were worked out by calculating correlation coefficients as per method described by Panse and Sukhatme (1967).
6. SUMMARY AND CONCLUSION

The present investigation was carried out in field as well as pot studies in Alfisols and Inceptisols soils. The field study was undertaken in order to characterize different phosphorus fractions in rhizosphere as well as in non-rhizosphere soils under Maize, Sorghum, Mustard and Wheat. However, the pot study was undertaken with the aim to differentiate the effect of plants, microorganisms and phosphatic fertilizers on different soil phosphorus fractions under different plant species namely, Maize, Sorghum, Mustard and Wheat in Alfisols and Inceptisols. The summary of the results are as follows:

1. Depletion of available soil phosphorus after 45 DAS with time was significantly higher under Alfisols than Inceptisols. The effect of plant species on available soil P was in the order of maize>sorghum>mustard>wheat crops.
2. Inorganic P had increased (8.46 to 41.84%) in Alfisols and (9.91 to 12.78%) in Inceptisols under all the plant species.
3. Adsorbed phosphorus differed with particular soil type at different growing stage of plants.
4. The buildup of calcium phosphate was due to combined effect of crop, microbes and fertilizer. Significant differences were observed among the soil type and between fumigation levels.
5. The fixation of soil phosphorus as Al-P was numerically higher for Alfisols than Inceptisols.
6. The transformation of soil phosphorus in Fe-P was considerably higher in Inceptisols than that in the Alfisols. The buildup of Fe-P ranged from 2.95 to 11.48% in Alfisols and from 14.15 to 19.99% in Inceptisols.

7. Treatment combination of soil types and fumigation levels had specifically influenced the occluded Fe-P at specific crop stages and it was specific to plant species within individual soil type.

8. An appreciable increase in occluded Al-P under different plant species was observed with highest Al-P under maize.

9. The effect of plant roots along with microorganisms (B₃ and B₅) appreciably altered the dehydrogenase activity of the soil.

10. Under native soil condition (without fumigation) soil biomass was significantly altered by different plant species which was more between 30 and 45 DAS of plant age.

11. Acid phosphatase activity decreased with increase in crop growth period up to harvest due to rooting behaviour as well as secretion pattern of roots and decaying of roots with age of plant.

12. Changes in inorganic P between rhizosphere and non-rhizosphere soils for individual soil types were found at par for both the soil types. These changes in inorganic P transformation under different plant species at varying growth stages would be correlated with root morphology, rhizodeposition as well as microbial activities in the soil.

13. The rhizosphere effect of different plant species on adsorbed P was inconsistent and varied with soil type and growth stages as well as plant type. This could be attributed due to the organic chalets secreted by roots and their interaction with
microbial transformation and ionic strength of the soil at prevailing pH in soil rhizosphere.

14. The buildup of Ca-phosphate was higher in rhizosphere than non-rhizosphere under all the plant species. The effect of different of plant was found to be in order of maize>sorghum>mustard>wheat at 45 DAS.

15. Change of Al-P was differed between rhizosphere and non-rhizosphere of individual soil type was at par, except 45 DAS in maize and at harvest in sorghum.

16. Higher content of biomass carbon at 45 DAS and harvest than initial status is an indication of good microbial soil health for better P-mobilization in both the soil types. Crops having more root length density and surface area favour the improvement of biomass carbon.

17. The change as well as buildup of Fe-P was higher in Alfisols than Inceptisols, 2/3rd of increased or buildup at 45 DAS and 1/3rd buildup was fixed up to harvest irrespective of crops.

18. The occluded Fe-P content as well its buildup in the soil was higher under Inceptisols than Alfisols regardless the plant species at both 45DAS and at harvest.

19. Rhizosphere and non-rhizosphere soil was found at par towards occluded Al-P under individual soil type for all plant except 45 DAS under maize in Alfisols and mustard in Inceptisols. The buildup of occluded Al-P was continued till harvest of the crops.

20. More dehydrogenase activity was observed under rhizosphere than non-rhizosphere which would be attributable to root effects of crops.
21. The effect of plant growth on P-use efficiency was considerably higher (5-15%) under Alfisols as compare to Inceptisols. Fumigation trend was higher in order to $B_5>B_4>B_3>B_2$ (Plant+MO+Fertilizer effect $>$ Plant + fertilizer effect $>$ Plant + microorganism effect $>$ Plant effect) Treatment $B_4$ (Plant + fertilizer effect) and $B_5$ (Plant+MO+Fertilizer effect) significantly influenced the P-use efficiency as compare to $B_2$ (Plant effect) and $B_3$ (Plant + microorganism effect) due to fertilizer effect.

22. Application of P-fertilizer with and without microbes was non effective towards per cent P-recovery under maize and sorghum which was considerably higher under wheat and mustard.

23. The per cent yield response was higher in Inceptisols as compared to Alfisols. The similar trend was observed under fumigation levels $B_4$ (Plant + fertilizer effect) and $B_5$ (Plant+MO+Fertilizer effect) as compared to $B_2$ (Plant effect) and $B_3$ (Plant + microorganism effect) irrespective of crops. It means the application of inorganic P-fertilizer response towards yield was considerably higher as compared to non-application of fertilizer.

24. The available phosphorus was almost double in Inceptisols than Alfisols. The same trend was found in rhizosphere soil due to plant roots induced changes in rhizosphere compare to non-rhizosphere. Available phosphorus response was higher in wheat, mustard then maize and sorghum due to difference in their P-requirement and capacity to mobilize native soil phosphorus.

**Conclusion**

The results obtained during the course of present investigation may be concluded that the soils under study (i.e., Alfisols and Inceptisols) are considerably differed in soil
phosphorus status, soil enzyme activities, soil biomass carbon, biomass phosphorus as well as different inorganic phosphorus fractions with higher under Inceptisols except iron and occluded iron phosphorus fractions which are higher in Alfisols. The transformations of different soil inorganic P (Pi) fractions were significantly altered by growing of different plant species with higher under cereals (i.e., Maize, Sorghum, and Wheat) followed by oilseeds (i.e., Mustard). Generally these Pi fractions were higher under rhizosphere than non-rhizosphere, although the difference was non-significant. The quantitative significance of plants, microbes and phosphatic fertilizers on different Pi fractions were clearly indicated that highest changes were observed under application of phosphatic fertilizers followed by microorganisms and plants. Further, the changes in soil enzymatic activities (i.e., dehydrogenase and acid phosphatases), soil microbial biomass carbon, and phosphorus were highest under microbial effect (i.e., treatment B₃ and B₅). However, the per cent yields response, per cent phosphorus recovery and phosphorus use efficiency were significantly (P=0.05) higher under application of phosphatic fertilizers alone or in combination with microorganisms (i.e., treatment B₄ and B₅). The study on phosphorus influx in different crops indicated that highest P-inflow was recorded up to 30-45 DAS and drastically reduced thereafter up to crop harvest. This P-inflow rate was specific to plant species as well as the cropping season which was higher for winter season crops then kharif season crops. Further on critical assessment of the results it was clear that phosphorus influx depends on root architecture, morphology as well as soil P status and the prevailing soil conditions which were generally higher in Inceptisols then Alfisols. Generally despite of higher P status of Inceptisols the phosphorus dynamics during crop growth was considerably higher in Alfisols.


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