CHAPTER I

INTRODUCTION
INTRODUCTION

Soil environment is ever changing due to interactions between the plants and the microorganisms within it. It is really very interesting to note the interactions between the plants and microorganisms within the soil. The interactions between plants and microorganisms mainly signify effect of the plants on the microorganisms and on the other hand effect of the microorganisms on the plant. As Hiltner defined in 1904 this region of contact between plant roots and the microbes - the rhizospheres is a topic of great importance to discuss. The rhizosphere of different plants inhabit different groups of microorganisms and that too is influenced in a diverse way by different factors. Generally greater number of microorganisms are found in the rhizosphere than in the soil away from the roots.

The relationship between the plant roots and the soil microorganisms has been investigated by many scientists. It is now eighty years since Hiltner observed that the soil adjacent to the plant roots, that is, the rhizosphere harbours a more pronounced microbial population than the soil away from the roots. Since then
many workers have been shown the qualitative and quantitative differences between the microflora of the rhizosphere and the distant soil. With the advent of new techniques, the intricate relationship between the plant roots and the soil microorganisms is more elucidated.

The microorganisms in the rhizosphere may be influenced by many factors, such as, stages of growth of a crop - its nature, type of soil, seasonal variation, soil treatment etc. These factors actually affect the plant or the crop which in turn result in the change of equilibrium of the microbial flora, in contact or away from the plant roots.

LITERATURE REVIEW OF THE PRESENT WORK

The present work is confined to the study of -

1. Influence of stages of growth of the barley plants on the rhizosphere and non-rhizosphere microflora.

2. Influence of different soil pH on the rhizosphere and non-rhizosphere microflora of barley.
3. Influence of seasonal variation on the microflora in the rhizosphere of barley and in the non-rhizosphere soil.

4. Relationship between seed borne fungi and their colonization of roots.

5. Influence of soil and plant treatment on the rhizosphere and non-rhizosphere microflora.

6. The rhizosphere in relation to the nutritive requirements of the soil bacteria.

Review of Literature:

A. Qualitative and quantitative studies of microorganisms in rhizosphere and non-rhizosphere soils.

I. Influence of stages of growth of a crop on the rhizosphere and non-rhizosphere soil.

II. Influence of different soil pH on the rhizosphere and non-rhizosphere microflora.

III. Influence of seasonal variation on the rhizosphere and non-rhizosphere microflora.
IV. Influence of soil and plant treatment on the rhizosphere and non-rhizosphere microflora.

B. Nutritional requirements of the predominant microbial flora in the rhizosphere of a crop.

C. Relationship between the seed-flora and the rhizosphere microflora.

A. Qualitative and quantitative studies of microorganisms in the rhizosphere and non-rhizosphere soil: - It is well established that soil in the immediate vicinity of plant roots (rhizosphere) contains larger number of microorganisms than soil more distant from the roots. Besides the quantitative differences, there also exists qualitative differences between the rhizosphere and non-rhizosphere microorganisms. In the early years of soil microbiology most of the published work concerned mainly with the quantitative aspects. With time microbiologists have paid attention towards the qualitative aspect of the rhizosphere microflora as well.

Peterson, (1958) observed that soils differing in pH affect the predominance of certain fungi on corn roots. Information pertaining largely to fungi which are
in an active mycelial state, has been obtained by examination of the fungal populations of thoroughly washed roots of wheat, beech seedlings and rye grass. Light intensity has been found to exert a marked effect on the composition of the fungal flora of beech seedling roots, (Peterson, 1958).

I. Influence of stages of growth of a crop upon the rhizosphere and non-rhizosphere microflora:

Katznelson, Lochhead, and Timonin (1948) were of opinion that in general most of the plants did not exert a great effect at the early stages of growth and the maximum effect generally appeared after the plant had attained appreciable size, reaching the limit of vegetative growth. As the plants start to degenerate a rapid fall in the numbers of bacteria occurred. Graf (1930) observed a quantitative increase at the root surface during the period of growth followed by a decline during the ripening period and a further decline after harvest. Manzon (1936) in a study of a variety of leguminous and non-leguminous plants, concluded that the numbers and activity of the bacterial population of the rhizosphere varied greatly, depending both on the species of the plant and on the phases of its development. Starkey (1929) did not observe any striking differences between the
microflora of mangel, beet, barley, maize, rape, vetch and soyabean except a quantitative increase as the plants matured. Katzenelson (1946) was of opinion that the effect caused by any treatment must also be correlated with the stages of growth of the plant. He observed that the rhizosphere microflora was not prevalent in rapidly growing, vigorous mangel plants only at the height of growing season. Katzenelson, Lochhead and Timonin (1943) were of opinion that the abundance of microorganisms in a region indicated intense activity, in that particular region which was again influenced by stages of growth of a plant. In their studies with bacteria they observed that with most plants the increase in the rhizosphere effect was greatest at the height of the growing period followed by a decrease at senescence. Numbers of gram-negative, non-sporeforming rods are affected by nature and age of the plant. Starkey (1929) observed the occurrence of mitrifying bacteria being specially pronounced in the roots of plants in more advanced stages of growth. Krassilnikov et al (1933) noted an increase of aerobic cellulose decomposing bacteria in the rhizosphere of sunflower and soyabean with the gradual development of the plant.
With healthy wheat roots, Peterson (1958) observed that at four intervals from the seedling stage to maturity, total counts of fungi in rhizosphere soil increased markedly with plant age. Species of *Fusarium* were abundant on the roots, their relative incidence increasing with plant age but their numbers were usually found to be less in rhizosphere than non-rhizosphere soil samples. Species of *Penicillium* was found to be present both in the rhizosphere and non-rhizosphere samples throughout the experimental period. Their activity was more pronounced only at the seedling stage, because it was observed that relatively high proportions of these fungi could be sampled from the roots of 7 days after seeding, which declined in subsequent samples. Peterson (1958) from his experiments concluded that plant age appear to affect the nature of the fungal population associated with roots. The relatively high proportion of fungi, such as, *Penicillia* and certain species of Mucorales found on the root surface and with rhizosphere of wheat in the early stages of development may be attributed to the excretion of specific substances from the root during that period.

Parkinson and Thomas (1969) also observed rhizosphere effect at different stages of plant growth. Dwarf bean plants were grown under field conditions and
were sampled at six stages of growth - two leaf stage, four leaf stage, onset of flowering, late flowering, early pod formation, pod maturity and senescence. It has been observed that the lengths of mycelium in each of the rhizosphere samples was greater than in the non-rhizosphere sample and there is some indication of an increase in the length of mycelium in the rhizosphere with increase of plant age. However, the mean lengths of mycelium in rhizosphere soil from plants at 3, 5, 7 and 12 days after germination was only slightly greater than that in non-rhizosphere soil, and there is no statistically significant difference between the lengths of mycelium, the values recorded for rhizosphere soil from 9 and 20 days old plants did show a statistically significant increase in the lengths of mycelium compared to the non-rhizosphere soil.

From their experiment it has been observed that at each sampling time the mean lengths of mycelium in the non-rhizosphere soil samples showed less fluctuation throughout the sampling period than did those of the rhizosphere samples. It has also been observed that the lengths of the mycelium in rhizosphere soil increased with increasing plant age to a maximum at the stage of
pod maturity. At senescence there was a marked decrease in the lengths of mycelium in the rhizosphere soil.

Rovira, 1965 observed that the oxygen uptake by rhizosphere soil samples increased with increasing plant age to a maximum at the late flowering - early pod formation stage. After this a rapid fall in the oxygen uptake by rhizosphere soil samples was observed, there has also been observed a wide variation in Azotobacter numbers in the rhizosphere of wheat at different sampling periods.

Plants of different ages exerted different effects on the nitrifiers. Riviere (1959) for example, found an increase at tillering and flowering of wheat while Molina and Rovira found a definite stimulation of nitrosomonas and nitrobacter around the roots of maize and lucerne at the age of 15 days but not at 46 days (1964). Rovira (1965), in his studies observed that the frequency of isolation of fungi from root segments increased with the age of the roots. He concluded that in general the number of organisms in the rhizosphere and the rhizosphere plane, estimated on either a soil wt. or root wt. basis, increased with age of the plant. Riviere (1959) found that wheat gave a maximum rhizosphere effect at the end of
tillering when ear formation was occurring, but after ripening the effect diminished.

Strzelczyk, 1966 carried on experiments with wheat, radish and onion to study the development of antagonists of Azotobacter in the rhizosphere of plants at different stages of growth in two soils. According to him the fact that some plants exert a greater effect on numbers of antagonists at certain stages of growth than others in the same soil implies that root excretions or related phenomena may play a very important role.

King and Wallace, 1956 observed that with regard to the age of the plants the proportions of Gram-positive rods in rhizosphere and control soils together were distinctly greater for mature barley than young barley plants. The proportional occurrences of bacteria that formed acid from glucose seemed to be altered only in relation to the age of the barley plants; their numbers increased in the rhizosphere sample of mature barley plants. The age of the plants did not seem to be related to the occurrence of the starch-hydrolysing bacteria. The proportional occurrences of nitrate reducing bacteria were greater in the rhizosphere sample of the young plants than in those of the mature. The percentage occurrence of gelatin liqueying bacteria were greater in control soils
than in rhizosphere soil and the difference was greater with regard to oats than with barley. Furthermore, the occurrences of these bacteria in the control and the rhizosphere soils of young barley were about the same whereas they differed significantly in the control and rhizosphere soils of young oats. The results are not in general agreement with those of Lochhead (1940) who showed that a greater percentage of liquefying bacteria occurred in rhizosphere soils of tobacco and flax than in control soils; an exact comparison cannot be made, however, because of the different soils and crop plants.

R.K. Saksena, 1969 was of the opinion that age of the plant and the environmental condition profoundly influence the microbiology of the rhizosphere region. Pimonin, 1940 recorded the establishment of a rhizosphere microrlora within these days of seed germination. Further development of rhizosphere population depends on the growth pattern of the plant. Usually maximum activity in the rhizosphere region is reported when the vegetative growth is at its peak.

Rovira, 1959 found that light intensity, temperature and age of the plant had a marked influence on the amount and pattern of amino acid exudation by
Clover and Tomatoes. Voroshilov, 1956 reported that the largest number of fungi and bacteria occurred in the rhizosphere of apple during flowering and fruiting, whereas fungi alone occurred in great numbers during shoot growth.

Kagti, 1966 was of the opinion that the relative incidence of microorganisms occurring in the rhizosphere of wheat from the time of seed-germination to maturity and in control soils indicated that the quantitative nature of the soil microflora markedly influenced by the growing plant. In the rhizosphere soil of young and mature plants species of *Mucor*, *Mortierella*, *Pythium* and *Penicillium* were relatively more abundant. On the other hand species of *Fusarium*, *Cylindrocarpon*, *Trichoderma*, and a number of sterile dark mycelial forms were isolated more frequently from the rhizosphere soil of older plants. He has also observed that the composition of the fungal flora associated with the roots changes with ageing of the crop. In general, succession in the young roots commences with a population dominated by species of *Mucor*, *Mortierella*, *Pythium* and *Penicillium*. In late maturity and in old roots this is superseded by a number of fungi consisting mainly of *Fusarium culmorum*, *Trichoderma viride*, *Cylindrocarpon radicicola*, and a number of sterile dark mycelial forms. The occurrence of
species of *Penicillium* and *Horticerella* remains fairly constant throughout the life of the crop. Chan Katznelson and Rouatt 1963 were of the opinion that a significant feature of the rhizosphere microflora was that the age of the plants played a far greater role in affecting differences in the bacterial growth patterns than the kind of the plant.

II. Influence of different soil pH upon the rhizosphere and non-rhizosphere microflora:

The influence of different pH of the soil on the rhizosphere microflora has been observed by many workers, but it is not yet clear whether the effect is direct or it is through the influence of the soil on plant growth.

In a study of corn plants growing in seven different soils (pH varying from 4.5 - 8.1) Thom and Himmel (1931) noted that the number of fungi on the corn roots was greatest in the most acid and in the most alkaline soil. Actinomycetes were not affected by soil differences. The number of bacteria was greater on the roots grown in soil slightly acid or neutral. Species of *Trichoderma* predominated in the roots in acid to neutral soil whereas in the alkaline soils *Penicillia* predominated.
Brian, Hemming and McGowan (1945) observed the prevalence of species of *Penicillium* in acid soils of Warenam Heath. Katznelson and Richardson (1948) found that certain fungi of "pathogenic capability" such as *Cylindrocarpon*, *Cylindrocladium* and *Rhizoctonia* were more suitable to an acid reaction than common saprophytic fungi. Rishoeth (1949, 1951) while investigating *Fomes annosus* root rot of Pines, found *Tricnoderma viride* to be far more abundant in acid than in alkaline soil. Warrup (1951) observed that in grass land soil most of the species of *Penicillium* were found in the very acid soils and they were comparatively rare in the alkaline soils. Garrett (1956) found that Fusarium wilt caused by various biotic forms of *Fusarium oxysporum* tend to be favoured by acid soil conditions. Peterson (1958) working on wheat and red clover grown in different soils observed more pronounced rhizosphere effect in a relatively infertile sandy soil than in a more fertile clay soil. He found that species of *Trichoderma*, *Penicillium* and *Cephalosporium* were stimulated in the rhizosphere of wheat grown in acid sandy loam, whereas species of *Gliocladium* were stimulated by wheat roots grown in alkaline chalk soil. He also reported that predominance of *Fusarium oxysporum* on the root surface of red clover was favoured by acid sandy soil whereas alkaline chalk soil was found to favour the incidence of *Cylindrocarpon* on the root surface. The rhizosphere effect in
nearly neutral clay soil appeared to be somewhat inter-
mediate. In such soils both *Fusarium* and *Cylindrocarpon*
were found to occur on the root surface in approximately
equal proportions.

Kagti, 1969 in a study of the influence of —
soil types on the rhizosphere effect noted more pronoun-
ced rhizosphere effect in young wheat plants grown in
acid sand stone soil (pH 6.2). In mature and fully
developed wheat plants, however, greater rhizosphere
effect was observed in acid moorland soil (pH 3.5).
Species of *Penicillium* and *Trichoderma viride* were
recorded in greater abundance in the rhizosphere in acid
moorland soil, whereas *Cylindrocarpon radicicola* and
*Fusarium culmorum* were found to be widely prevalent in
the rhizosphere in alkaline peat soil and alkaline
limestone soil. *Fusarium culmorum*, *Cylindrocarpon*
*radicicola* and *Trichoderma viride* were found to occur in
fairly large numbers in acid Keuper marl soil. The
occurrence of *Fusarium culmorum* and *Cylindrocarpon*
*radicicola* in fairly large numbers in the rhizosphere of
acid Keuper marl soil may probably be influenced by the
physico-chemical changes in the rhizosphere zone brought
about by the activity of the living roots. Species of
*Mucor* and *Mortierella* were very frequently isolated from
the rhizosphere in acid keuper marl soil. *Gliomastix convoluta* was found to be more prevalent in the rhizosphere in alkaline limestone soil. Thus soil types appear to influence the quantitative nature of fungi occurring on root surface at various stages in the life of the crop.

Katznelson, Lochhead and Timonin, (1948) were of opinion that the influence of factors such as type of soil, moisture content, soil reaction, treatment and temperature were of great importance to be studied. In a study of corn growing in different soils varying from pH 4.5 to 8.1 bacteria were found to be more numerous under slightly acid or neutral conditions, fungi were abundant on roots in the most acid and most alkaline soil, whereas, actinomycetes were not affected by soil differences.

King and Wallace (1956) concluded that the proportional incidences of some physiological groups of soil bacteria are not greatly increased, but in some instances are decreased by the presence of barley or oat roots growing in chicot sandy loam.

Peterson (1953) studied the influence of three different soil types on the rhizosphere flora of
Red clover and wheat. Soil type did not appear to influence total numbers of fungi in the rhizosphere of red clover; distinct differences were noted in that of wheat, highest counts being recorded for the light sandy soil. It is evident that soil type exerted a distinct effect on the distribution of certain fungi, not only in the soil but also on the roots and in the rhizosphere. Species of Mortierella and Gliomastix were found to be more prevalent in the alkaline chalk soil than in either the acid sandy loam or nearly neutral clay loam; whereas Trichoderma species were found only in samples from the two latter soils. The apparent stimulation of Trichoderma and Cephalosporium only in the rhizosphere of wheat grown in the acid sandy loam and of Gliocladium in the rhizosphere of wheat from the alkaline chalk soil further shows the influence of soil type. Species of Penicillium were abundant in rhizosphere and non-rhizosphere samples from both the sandy and the clay soil but were comparatively rare in corresponding samples from the chalk soil.

Rovira (1965) from his studies on interrelationships between plant roots and soil microorganisms concluded that the dominance of a particular fungal species on the root surface is influenced by the soil and there is
evidence that the bacterial flora associated with roots is affected by soil pH. One of the extreme examples of the influence of soil and environment on the rhizosphere effect was shown for desert plants which have R/S ratios of over 700 for bacteria. Apparently the roots provided microbial food stuffs and some buffering capacity against the desiccation of the desert soil.

E. Strzelezyk, (1966) observed that the rhizosphere : soil ratio for actinomycetes is higher in Grandby Sandy loam than in upland soil, whereas the reverse effect may be observed with bacteria.

III. Influence of season on the rhizosphere and non-rhizosphere microflora:

In a given soil environment, the components of the microbial population are in equilibrium with one another. Any change in this environment produced by season, growing crop or soil treatment may shift this balance to a new one which is a reflection of the biological activity of the new factor.

H.G. Gyllenberg, (1957) observed that in field experiments with oats the composition of the
Bacterial population in the rhizosphere was found to be almost stable during the whole period of plant development from young seedlings to maturity. In the beginning of the growth season the soil flora was quite different from that of the rhizosphere. It was, however, successively changed and towards the end of the season, it became similar in composition to the rhizosphere population.

Ramachandra Reddy, (1968) observed that foliar sprays of different nutrients and antibiotics resulted some change in the rhizosphere microflora but the type and magnitude of changes depended upon the nature and concentration of substances used, as well as season of the crop growth.

Katznelson and Chase, (1944) carried on experiments to observe seasonal variation in the rhizosphere and non-rhizosphere population. They were of opinion that the microbiological equilibrium is disturbed by seasonal change. Their results obtained over a period of 21 months represent the bacterial response to the combined effects of season, crop and soil treatment. Organisms with very simple food requirements were very prominent in the fall and winter of 1940, declined in
the summer of 1941 and tended to rise again in the following fall and winter, whereas bacteria which require yeast extract varied more or less conversely. Bacteria requiring known amino acids or growth factors showed some fluctuation but none could be correlated with change of season. On the other hand, organisms growing only in soil extract semisolid agar fluctuated regularly with the season, being less in the late fall and winter and high in the early spring and summer.

IV. Influence of soil and plant treatment on the rhizosphere and non-rhizosphere microflora:

Katznelson, Lochhead and Timonin (1948) were of opinion that soil treatment does not appear to affect numbers of organisms in the rhizosphere. Liming was reported, however, to increase numbers by 25%. Manuring, though invariably inducing large increase in the microbial population of the soil did not appear to exert a similar effect on the root population. The rhizosphere of plants in soil treated with acetic acid supported a red-green form of *Penicillium* (20%), *Verticillium* (28%) and *Cladosporium* (4%) whereas the soils showed none of these but contained *Aspergillus* (6%), *Trichoderma* (12%) and an unidentified fungus (24%) and other forms such as *Mucor* and *Fusarium*. 
Katznelson and Chase (1944) studied the influence of soil treatment together with that of season on the incidence of nutritional groups of bacteria. Their conclusion is that in a given soil environment, the components of the microbial population are in equilibrium with one another. Any change in environment produced by season, growing crop or soil treatment may shift this balance to a new one which is a reflection of the biological activity or the new factor. This applies to the interrelationships not only among broad groups of microorganisms such as protozoa, fungi or bacteria but also among the constituent members of these groups. To what extent the new equilibrium will persist depends on the quantitative and qualitative intensity of the new environmental factor and the length of time over which it operates, because it is obvious that the inherent soil properties which are the results of thousands of years of adaptation will not be altered appreciably by a superficial treatment applied over a comparatively short period of time.

Taylor and Lochhead (1933) made investigations on a non-selective basis, of the qualitative nature and relative incidence of the different types of the bacterial flora of three soils differing in fertility. Inspite of unequal productivity, the soils showed no outstanding
differences in the relative incidence of the bacterial groups.

Many workers in recent years have envisaged the possibilities of improving the plant growth and crop yield by modification of rhizosphere microflora. In a number of cases the rhizosphere microflora has been altered by giving certain treatments to the plant. Halleck and Cochrane (1950) observed changes in the rhizosphere microflora of bean plants which were sprayed with bordeaux mixture. Ramachandra Reddy (1959) while studying the rhizosphere microflora of rice, observed that urea sprays modified the rhizosphere environment by exerting a change both in nature and amount of root exudates which in turn possibly influenced the microbial balance. It was also reported that zoliar spray of certain hormones like indole acetic acid or propionic acid resulted increase of rhizosphere population.

Milton H. Schroth and Floyd F. Hendrix, Jr., (1962) observed that chlamydospores of *F. solani* *F. phaseoli* germinated when alfalfa hay and cut straw or rye, wheat and barley were incorporated into the soil.
Louw and Webley (1959) reported that maximum number of bacteria were found in the rhizosphere and rhizoplane of plants supplied with superphosphate. Besides the increase in total rhizosphere population the numbers per unit length of root also increased, indicating a greater leakage of nutrients from roots of well-fertilized plants. Clark (1949) put forward the theory that the responses observed in the rhizosphere microflora following soil treatment result from the effect of plant growth rather than any direct effect on the microflora.

N. Relationship between seed-borne fungi and their colonization of roots:

On a study of seeds of certain agricultural crops, Wallace and Locnhead (1951) postulated that rhizosphere organisms constitute a group morphologically, physiologically and nutritionally intermediate between the indigenous soil bacteria and epiphytic seed microflora. Rovira (1965) is of opinion that seedflora could establish themselves on the root surface. Sathe and Subramanyam (1931) reported that microorganisms associated with seeds or a given crop are derived from the soil and that none or them is specific to any of the seeds. Hofer and Hamilton (1940) also reported that the species of bacteria
observed on seeds are similar to those commonly found in soil. Peterson (1959) studied the relationship between the seedborne fungi and their colonization of roots of barley, rye and wheat. His results showed that the main types of fungi present on the seed coat were either entirely absent or their proportions were very low in the rhizosphere flora. In conclusion he wrote that soil is the primary source of fungi which colonize the roots of healthy plants. The studies of Peterson (1959) and Parkinson, Taylor and Pearson (1963) have suggested that the initial seedling root surface flora is not derived from that of the seed coat. Parkinson and Clark (1964) have found that several fungi isolated from the seeds of shallots and garlic colonized adventitious roots of these plants growing in sterile medium. Kagti and Barueh observed a marked similarity between the fungi associated with rice grains and the fungi commonly found in soil.

VI. The Rhizosphere in relation to the nutritive requirements of the soil bacteria:

One of the important groups into which soil bacteria may be divided on the basis of nutritional requirements comprises those organisms for which amino acids are needed for growth (Locnhead and Chase, 1943). This group appears to be significantly related to crop growth, for
studies with a variety of plants have shown that bacteria requiring amino acids are preterentially stimulated in the rhizosphere (Locnhead and Tnexiton, 1947; Wallace and Locnhead, 1949). These results suggested that the plant, through the agency of root excretions, might play an important role in the nutrition of the rhizosphere forms. In addition to difference in the relative occurrence of various morphological types in the rhizosphere compared to normal, there might also be significant differences in the nutritive requirements of the organisms in the two groups. For an understanding of the interrelationships between plant roots and the soil microflora, one should observe the physiological activity and nutritional requirements of the organisms aggregating at the root-soil interface. It was pointed out by Thom (1935) that most of the organisms associated with the rhizosphere belong to species active in the decomposition of fresh organic matter and not to forms involved in the breakdown of humus residues in soil. By means of plating on a selective medium and isolation, Locnhead (1940) found a more active bacterial flora in the rhizosphere than in the soil apart from the root. A more extensive differentiation of the nutritional groups of bacteria was proposed by Locnhead and Chase (1943) which involved a determination
of growth response in 7 cultural media of increasing complexity. In an application of this procedure to a study of mangels, it was found that bacteria with simple requirements and those responding to amino acids were preferentially stimulated in the rhizosphere; (Katznelson, Lochhead and Timonin, 1948). The classification of soil and rhizosphere bacteria on nutritional requirements has shown that most rhizosphere isolates grown on the basal medium of glucose, nitrate and salts required amino acids, while most soil isolates require complex growth factors supplied by soil extract and yeast extract. Their findings suggested that amino acids may be one of the major nutrients of the rhizosphere microflora. Cook and Lochhead (1959) found that many amino acid requiring bacteria from the rhizosphere synthesized thiamin, biotin and vitamin B₁₂, which explains why many vitamin requiring bacteria occur in the rhizosphere although many root exudates are deficient in the B vitamins.