CHAPTER IV

DISCUSSION

4.1. Soil studies:

From the conventional soil analysis (Table-2), soils of group-I section showed medium in organic carbon and low in available potash content. On the other hand, rest of the soil groups are comes under high fertility range in respect of available potash and organic carbon content, and among them group-II section possess highest fertility followed by group-III and group IV section respectively.

As regard total nitrogen determine by sulphuric acid digestion method group-II and III section contains almost equal and higher values compared to group-I and IV section. However, as per salicylic + sulphuric acid digestion method, soil group I and IV showed higher values of nitrogen compared to group II and III. Inorganic nitrate and nitrite nitrogen in all the soil groups are very poor and almost equal. From the above analysis of nitrogen it is evident that nitrogen in soil group I and IV, are present, in more complex form compared to soil group II and III.
Considering the organic carbon Available notash and nitrogen over all fertility status is high in soil group III followed by group II, IV and I respectively.

pH estimation of soil samples during different month of a year (Fig. 1) showed that, soil group IV (virgin section) exhibit fluctuation of pH between 5 to 5.8 with an average pH of 5.3 virgin section also maintain a higher pH in all the month of the year compared to rest of the soil groups. Average pH of soil group-1 was found 4.5 while for soil group II and III it was recorded 4.2.

Fluctuation of pH, however, more in group-I compared to group II and III section and it was in the range of pH 4.2 to 5.3. Between soil group II and III fluctuation of pH is more in group II while soil group III maintain a steady state pH throughout the year. Fluctuation of pH among different soil groups reflected the intensity of Agrochemical used in the soil (Table-7). In virgin soil which does not receive any agrochemical thus maintain a stable pH. Similarly soil group I and II received wide array of agrochemical hence showed high fluctuation of pH compared to soil group III which received less agrochemicals. Fluctuation of pH is due
4.2. Transformation of Fertilizer nitrogen:

Results of transformation study of different fertilizer nitrogen (Table 2 and 4) clearly indicate that nitrification, denitrification and ureolytic properties of different soil groups differ significantly. Higher recovery of nitrate-nitrogen in ammonium sulfate amended samples after 24 hours incubation of soil group-I indicate that nitrification rate is high in this particular group. Nitrification rate of subsequent group decrease in the order of II, IV and III respectively. However, result after 72 hours of incubation showed that in soil group-II rate of immobilization is also very high and hence no nitrate was detected after 72 hours of incubation. Soil group II on the other hand gives highest recovery of $\text{NO}_3^-$ after 72 hours indicating its lowest immobilization rate.

Poor recovery of nitrogen in the nitrate amended samples (Table 2b) indicates nitrate either immobilized or lost through denitrification. Higher nitrite accumulation at 72 hours compared to 24 hours incubation is not due to nitrate reduction, because if it so almost
equal amount of $\text{NO}_2^-\text{N}$ would have recovered after 24 hours also. Therefore it is clear that nitrate was first immobilized and then nitrite formation occurs, probably following the death and lysis of cells. Since nitrate is a common intermediate to both nitrate assimilation and denitrification (Alexander 1961). Recovery of ammonia after 24 hours of urea transformation study (Table 3.b) appears that except group I, rest of the soil groups are high in urease activity. Higher recovery of ammonia after 72 hours of incubation in soil group I of poor urease activity, compared to other soil group, can be explained on the basis of reappearance of immobilized $\text{NH}_4^-\text{N}$ probably through dead and lysis of cells. Poor recovery of ammonia in virgin section at 72 hours may be due to ammonia volatilization and immobilization.

Volatilized $\text{N}$ in the potassium permanganate vial (Table 4) obviously related to the quantity of $\text{NH}_3$ formed. From this Table it is clear that $\text{NH}_3$ formation occurred in the presence of all the three chemicals. However, the high values of $\text{NH}_3$ in the urea amended samples obviously indicate that considerable quantity of N is volatilized as $\text{NH}_3$ following ureolysis. Soil group IV
showed higher volatilized \( \text{NH}_3 \) followed by soil group III, I, and II respectively. Although the values of volatilized-\( N \) in much lower in the potassium nitrate and ammonium sulphate samples it can not be assumed that nitrogen volatilization was low in the presence of these chemicals. Failure of detection of nitrate in the potassium nitrate and ammonium sulphate amended samples clearly suggest that nitrogen volatilization must be taking place through \( N_2, N_2O \) and \( N_2 \) formation.

"Volatilization during nitrification as reported by Nelsen and Schmidt (1978) and nitrogen volatilization through nitrate respiration as reported by Alexander (1961) are therefore to be taken into consideration."

The \( pH \) readings of the samples amended with the various chemicals (Table 5) obviously donot related to the quantity of \( \text{NH}_4-\text{N} \) detected in the soil samples. Had this been so, then, the samples of ammonium sulphate amended samples, which showed higher values of \( \text{NH}_4-\text{N} \) than urea amended samples in both the 24 hour and 72 hour samples would have shown increase in \( pH \) value. On the contrary, the \( pH \) increase occurred only in the urea amended samples. Therefore, it is clear that \( pH \) increase observed
is due to \( \text{NH}_3 \) released rather than \( \text{NH}_4^-\text{N} \) accumulated in the soil. Since values of volatilized-\( \text{N} \) in the \( \text{KMnO}_4 \) vials in the ammonium sulphate amended samples is very low, it is obvious, that \( \text{NH}_3 \) formation did not occur in the presence of ammonium sulphate. In the urea amended samples, the pH rise which were 8.5 and 8.7 in group I and IV (Table 5) more or less proportionate to the quantity of \( \text{NH}_3 \) detected which was 8.9 ug and 13.75 ug (Table 4) respectively. Likewise, little rise in pH of urea ammended group II and II, samples is due to less evaluation of \( \text{NH}_3 \) compared to group I and IV samples.

**Soil perfusion:**

Results of soil perfusion studies (Group I and III) with different solutions (Fig.2 to 7) showed that the elution pattern of respective chemical and product formation varies between soil group I and III.
Sterilization of the soil columns apparently does not eliminate completely the soil enzyme activity, while the quantities of ammonium, nitrite and nitrate (fig. 2) eluting in the sterile columns perfused with water are clearly present in the soil samples which get eluted with water. From this figure it appears that, nitrate in group I was not present, but nitrite was present in both groups I and III. Group III had higher content of nitrite than group I.

Ammonium - N values (fig. 3) in the urea perfused columns which are distinctly more than the elutes of soil columns perfused with water indicates that rapid ureolysis occurred both in sterile and unsterilized columns, while the quantities of ammonium in the sterile and unsterile columns of group III does not vary, group I samples yield enormously high values of ammonia in the unsterile columns. This may be attributed to the inability of the samples from group I to trap ammonium within the soil matrix, since soil incubation studies also indicated that the ureolytic activity resulting higher rate of \( \text{NH}_3 \) release in group I enhanced pH values, the microbial reactions taking place in these strains may be different from those of samples from group III. The ammonium - N values in the sterile columns which were comparable to those of the samples from group III shows that sterilization eliminates this microbial activity, which causes rapid ammonium leaching through the soil columns.
Nitrite values, which increased 10 fold over the column perfused with sterile water shows that rapid ammonium oxidation occured both in the sterile and unsterile conditions. Although the quantity of nitrite - N in both group I and III were higher in the unsterile columns, then in the sterile columns, indicating microbial ammonium oxidation, the quantities of nitrite in the sterile columns may have been through chemical reactions. Likewise, nitrate formation which also occurred in both the samples from groups I and III may be chemical in sterile columns. In the unsterile column the quantities which are slightly higher than the quantities in the sterile column further, supports the view that both chemical and microbiological nitrite oxidation occured.

Thiocorea (fig. 4), apparently induces the column to behave like sterile columns, since no striking differences between the quantities of either ammonium nitrite or nitrate could be detected in the sterile and unsterile columns, it may be possible that thiocorea inhibit all microbial activity responsible for ureolysis and nitrification. It is here interesting to note that in the presence of thiocorea the elutes of groups III samples yielded lesser quantities of ammonium, nitrite and nitrate than the samples from group I.
Ammonium sulphate (Fig. 5) perfused columns yielded 10 times more ammonium in the elutes of unsterile columns than in the sterile columns. Since ammonium sulphate was sterilized together with the soil columns placed in the container fused above the soil column it is possible that ammonium might have become entrapped within the soil matrix due to sterilization. This may be the reason for the low values of ammonium in the elutes. The lower values of ammonium found in the elutes of group I than compared ammonium sulphate perfused columns suggested that, ammonium retention in samples from group I is better when the chemical used is ammonium sulphate rather than urea.

Nitrite values in both group I and III in the ammonium sulphate perfused columns though comparable to the values in urea perfused columns, are distinctly higher. This indicates, that ammonium oxidation is higher when ammonium sulphate is the substrate. To further support this view, the nitrate values also increased 10 fold over the urea perfused columns. The quantities of nitrite and nitrate in the sterile columns which are much lower than the unsterile columns clearly represent chemical reactions, leading to nitrite and nitrate formation.

As before as in the case of urea, thiourea, brought about change in the elution pattern, also in the presence of ammonium sulphate (Fig. 6). Although the difference between the sterile and unsterile columns was considerable in the presence of ammonium sulphate, it can
be seen from Fig. 5 and 6 that the high values of ammonium in the eluates of ammonium sulphate perfused columns was not found when ammonium sulphate was used, mixed with thiourea. Sterilization of the columns which had been sterilized with the mixture of ammonium sulphate and thiourea as in the case of ammonium sulphate caused also here a sharp reduction in the ammonium - N values. It is however, interesting that the difference in the quantities of ammonium recovered in group I and III when perfused with ammonium sulphate (Fig. 5) disappeared in the presence of thiourea (Fig. 6).

Although the low nitrite values in unsterile columns, suggests inhibition to microbial ammonium oxidation by thiourea, the enormously high yield of nitrate in the eluates particularly in the samples from group III, suggest inhibition to nitrification may not have occurred. Since thiourea is known to inhibit autotrophic nitrifiers (Painter 1970), it is to be expected that nitrate formation in this case is through the activity of heterotrophic nitrifiers. Thus failure of thiourea to inhibit nitrification in the presence of ammonium sulphate, is an evidence to heterotrophic nitrification in these soils.

Glucose amendment to enhance the activity of heterotrophic nitrifiers (Fig. 7), eliminated the differences in the elution pattern observed between the samples
of group I and III when ammonium sulphate was perfused without glucose amendment. Glucose amendment clearly promotes the activity of other organisms which brings about changes in the elution pattern. Nitrate formed which was lower than the ammonium sulphate used as substrate, indicates that, denitrification might have become enhanced due to the presence of glucose. This amendment of glucose to promote heterotrophic nitrifiers cannot therefore be taken into account.

Influence of pesticides on nitrification, denitrification activity in the soil estimated using potassium nitrate, ammonium sulphate and urea as the nitrogenous substrate showed (Fig. 8.2) that the pesticides do exert an influence on these activities.

The recovery of ammonium, nitrite or nitrate or volatilized N which was invariably higher in the samples from virgin sections may or may not indicate enhanced microbial activity in these samples. Enhanced microbial activity might in fact immobilize the nitrogen through nitrate assimilation into the cells, under such circumstance the nitrogen cannot be extracted in the KCl extract. Thus lower values of N in the KCl extract would ensure. On the other hand, higher values of nitrogen in the KCl extract, whether, in the form of either ammonium or nitrite or nitrate indicate enhancement of the nitrogen availability. Thus it would appear, that material of the treatment
received, the nitrogen availability was more in the samples from virgin sections with samples from good reactions falling next to it.

In the nitrate amended samples, the recovery of nitrate, lower than the values in the control (Fig. 12, 13) indicates the influence of the pesticides in nitrate utilization. The 0.3% Paraquat, Biltox, Tedion and Thiodan treated samples incubated for 3 days showing similar values as those of controls, indicates that the nitrate utilization activity, was not influenced by these pesticides. Values lower than those of the controls as in the case of 2,4-D, Dalapon and Kelthane treated samples indicates that these pesticides enhanced nitrate utilization activity. The lower values of NO₃ recovered in the samples treated with the increased concentration of 2,4-D and Dalapon (Fig. 13) further confirm their influence on nitrate utilization activity. Higher quantity of nitrate, than those found in controls when concentrations were either 0.6 or 1%, is more likely to have come about through enhanced nitrification following degradation of the nitrogen containing herbicide. Influence of paraquat to enhance nitrification, has been reported by Bezbahak (1983). Thus the values of NO₂ which is higher than the controls in the paraquat treated samples at all concentrations (Fig. 16,17) may not indicate, nitrate reduction activity.
Ammonium extractable from the soil in Kel in the nitrate amended samples also indicates nitrate reduction activity taking place in the soil. However, while the lower values of nitrite in the presence of 2,4-D, Dalapon and Kelthane suggest influence of these pesticides in denitrification activity. Ammonium recovery in the KNO₃ treated samples need not necessarily indicate denitrification activity. Higher values of ammonium, than those, found in the controls, in the Dalapon treated samples, when the concentration of Dalapon was either 0.6 or 1% showed that more ammonium could be recovered, when the concentration of Dalapon was higher. Since at lower concentration, such an effect of Dalapon on ammonium recovery, was not present (Fig. 8), it is likely, that the higher yield of ammonium with high concentration of Dalapon (Fig. 9), is due to autoclavage of cells.

Volatile-N in the pesticide treated samples amended with potassium nitrate was not very different from those of the controls. However, the enhanced values of volatile-N in the 15 day samples amended with 2,4-D, Dalapon and Kelthane (Fig. 20 and 21) suggest enhancement in denitrification activity.

In the urea amended samples, ammonium in the Kel extract, indicates the level of ureolytic taking place in the soil, in the presence of different pesticides. From the Figs 10 and 11, it is apparent, that ureolytic, is
not strikingly influenced by the pesticides used. Likewise, ammonium in the ammonium sulphate amended samples, also suggest, that ammonium recovery from the samples, is not influenced by the pesticides. From Fig. 11, it is however interesting to note, that the 1% concentration of Dalapon sharply reduced ureolytic activity. Since the ammonium values in the ammonium sulphate amended samples do not diminish (Fig. 11), the low values of ammonium in the 1% Dalapon treated urea amended samples must be due to suppressed ureolytic activity. The reduction in the ammonium values, in the urea and ammonium sulphate amended samples (Fig. 10 and 11), with the increase in the incubation time, suggest utilization of the ammonium as a substrate for nitrification.

Active nitrification, both in the presence of urea, and ammonium sulphate, is strongly indicated, by the recovery of nitrite and nitrate from the samples treated with different pesticides. Since nitrate recovery, in the presence of Paraquat, 2,4-D, Dalapon, Slitox, Tedion, Thiodan and Kelthane (Fig. 18) is not strikingly different from those of the controls in the 3 days samples, it is obvious, that these pesticides do not influence ammonium oxidation activity at this concentration either in the presence of urea or ammonium sulphate. At higher concentration of Paraquat, Dalapon and 2,4-D, the influence on ammonium oxidation to nitrite is more in the Paraquat treated samples than in the controls.
Likewise, nitrate values in the presence of Paraquat, is also more than the controls in all the three concentrations of Paraquat tried. The inhibition to nitrification by 2,4-D, Dalapon and Kelthane is indicated not only in the lowering of the nitrite, but also in the lowering of nitrate values, both in the presence of urea and ammonium sulphate. The inhibition of 2,4-D and Dalapon to nitrification, is particularly marked by the sharply reduced values of nitrate both in the presence of urea and ammonium sulphate as in fig. 15.

The enormous difference in the values of volatilized N, obtained in the urea and ammonium sulphate amended samples showed, that urea amended cultures evolved NH\textsubscript{3} evolution, is more in the presence of Paraquat, Dalapon, Blitox and Kelthane. The higher values of volatilized-N\textsubscript{3} with increase incubation time, as in fig. 24 showed that NH\textsubscript{3} accumulation, continued for a long period of time. From the values of NH\textsubscript{3}, as in fig. 23 it is apparent that the pesticides 2,4-D and Dalapon, which initially suppressed ureolysis were over come at a later stage, either by the ureolytic organisms or by other organisms of the soil that helped to detoxify the two herbicides. Since no increase in nitrite or nitrate values were observed in the samples incubated for a longer time, in the presence of 2,4-D and Dalapon it can be stated, that nitrification inhibition by these herbicides continued to remain for a long period of time.
Nitrogen volatilization, in the presence of ammonium sulphate which was higher in the presence of potassium nitrate, also suggested, that nitrogen loss must have occurred as NH$_3$. As in the case of urea amended samples 2,4-D and Dalapon, suppressed NH$_3$ formation, which as before was gradually overcome with the increase in the incubation time. But as before, ammonium oxidation to nitrate or nitrite oxidation to nitrate did not increase with period of incubation.

When no fertilizers were used, recovery of ammonium in the soil samples indicated the original content of ammonium present in the soil and extractable in Kel. Fig. 8 and 9 indicated that ammonium values reduced in the controls with the increase of incubation period. It is apparent from fig. 16 and 17, that the ammonium in the soil became oxidized into nitrite, which was detectable both the 10 and the 15 day samples. However, since no nitrate was recovered from these samples (fig. 12 and 13), it is possible, that the nitrate formed was immobilized rapidly. Ammonium recovery, which was higher than the controls in the presence of 2,4-D, Dalapon, Blitox and Thiodan suggests that nitrogen fixation activity may be enhanced by these pesticides.

Paraquat apparently contributes also to ammonium values in the samples treated with Paraquat (Fig. 9). The ammonium-N values of the Paraquat treated samples, which reduced when the period of incubation increased was
accompanied by the increase in nitrite and nitrate values. In the 15 day samples, nitrite values diminished accompanied by a further increase in nitrate confirming the findings of (Bezbaruah 1983), that Paraquat enhanced nitrification activity.

Nitrogen volatilization in the absence of additional fertilizers detectable in all the samples amended with Paraquat, 2,4-D, Dalapon, Blitox, Tedin, Thiodan and Kelthane showed that all the pesticides contributed towards nitrogen volatilization. Enhanced values in the 15 day samples (Fig. 20) in the presence of 2,4-D, Dalapon and Kelthane further confirmed the influence of these pesticides on denitrification activity. While the high concentration of the pesticides, Paraquat, 2,4-D and Dalapon (Fig.21) appears to suppress nitrogen volatilization activity, the values found on the 15th day and 15th day samples, confirm the influence of these pesticides on enhancing nitrogen volatilization.

From the estimation of ammonium, nitrite and nitrate found in the surface layers of the soil samples sprayed with urea and in combination with pesticides, Paraquat, 2,4-D, Thiodan, Kelthane, Blitox and Dalapon (Fig. 24-34) indicate that nitrogen transformations occurred also in surface layers. Ammonium, representing ureolysis rate, appears to be enhanced in the presence of Dalapon, Thiodan and to a certain extent in Kelthane treated samples. The high values of ammonium in the Paraquat treated samples, may not represent
the ammonium arising from ureolysis alone because Paraquat contains nitrogen (Fig. 24). It is possible therefore, that reduction of the nitrogen from the degraded Paraquat molecule, contributes to the high value of ammonium in the samples. The response of the samples from the two groups to such a decomposition of the Paraquat molecule appears also to differ slightly (Fig. 26). The samples from group III apparently degrade Paraquat faster than the samples from group II. However, it cannot be ascertained by this experiment whether Paraquat enhanced ureolytic activity or that the pesticide molecule become degraded. The finding of Bezbarua (1963) on the influence of Paraquat on enhanced nitrification and a gradual increase of nitrite (Fig. 28) and nitrate (Fig. 32) observed here indicates that Paraquat assisted ureolysis and nitrification.

The disappearance of the ammonium, nitrite and nitrate from the surface layers of the samples treated with Dalapon, 2,4-D and Kelthane confirmed the earlier findings that these pesticides may contribute to nitrogen volatilization. The high values of ammonium in the 24 hour samples (Fig. 27) treated with Dalapon, disappeared totally after 48 hours. Thus indicating rapid nitrogen loss. Had the values of either nitrite or nitrate increased (Fig. 31) respectively in 48 hour samples, proportional to the decrease of ammonium in the 72 hour samples, only then, could Dalapon be considered as a positive
influence on nitrification activity. On the other hand, all the ammonium released following ureolysis appears to have been lost into the atmosphere. From the N-volatilization studies as depicted in Fig. 22–23 it can be seen that N-volatilization in the presence of Dalapon is particularly high in samples incubated for period of 15 days. The influence of Dalapon on enhancing nitrogen volatilization is thus confirmed. The suppression of Blitox to ureolysis (Fig. 26) is obviously overcome by the 4th day in the soil samples. Thereafter, the gradual increase of ammonium-N, may or may not be entirely due to ureolysis. Since Blitox treated samples without fertilizers (Fig. 8 and 9) accumulated ammonium in considerably high quantities, it is possible, that even here, the gradual increase in ammonium is due to the positive influence of Blitox on nitrogen fixation. Although the nitrite value of Blitox treated samples (Fig. 31) diminished despite the sharp increase of ammonium values (Fig. 29) during the same period the increase in the nitrate values (Fig. 34) shows that Blitox also enhanced nitrification. Since several heterotrophic strains, which show both nitrification and nitrogen fixation properties have been isolated by Bezbaras (1983) from these soils, it is possible, that active nitrogen fixation and nitrification occurred, here, with the exception of Blitox treated samples, ammonium, nitrite and nitrate diminished with time in all cases.
It is also apparent, that ammonium oxidation to nitrite occurs almost immediately from the ammonium released through ureolysis. The nitrate accumulation which increases to a certain period of time might diminish through immobilization. From the fig. 31-34 it can be noted that nitrate does not disappear from the surface samples as rapidly as that of nitrite and ammonium. It is possible that the nitrite formation following ammonium oxidation is also autooxidized to nitrate.

Recovery of ammonium, nitrite and nitrate in the different layers, both in the presence of urea and ammonium sulphate, showed, that the chemicals applied, undergoes transformation by the time they reached the bottom layers. The bottom layer which contains the same quantities of ammonium in the ammonium sulphate treated samples from both the group I and II, indicate that ammonium reaches bottom layers when applied in the form of ammonium sulphate in considerable quantities. In urea amended samples the quantity of ammonium recovered, representing the ureolysis rate, obviously indicates the extent of ureolysis occurring at the different layers. Thus, it appears from the data on table 7, that in the bottom and the top layers of group III and in the bottom and the middle layers of group I, active ureolysis takes place. Introduction of the pesticides obviously brings about changes in this system. In case of group I the top layer values
which increased from 350 to 609 μg in presence of 2,4-D, to 385 μg in presence of Dalapon and 574 μg in presence of Thiodan, indicate, that in the top layers ureolysis activity was enhanced by this pesticide. Likewise, the reduction of values in the middle layer from 1225 μg to 770 μg in the presence of all the pesticides indicates, the inhibition to ureolysis occurred in the middle layer in the presence of pesticides. Likewise, reductions in values from 700 μg in the bottom layer, to values below 630 also suggest, suppression through ureolysis. In case of samples from group III reduction in values from 1785 μg in the top layer to values below 700 μg and in the bottom layer from 2380 μg to values below 1050 μg in the presence of all the pesticides, also suggest suppression of ureolysis by the pesticides. In the middle layers of group III increase in values from 525 μg to values above 535 μg observed in the case of all the pesticides indicate that ureolysis was enhanced by the pesticides in these layers. Inhibition to nitrite is obvious in the presence of all the pesticides except that of Paraquat in samples from both groups I and III in all the layers.

Nitrite formation, likewise, was also suppressed in all the layers except in the presence of Paraquat.

In the ammonium sulphate amended samples, the influence of the pesticides in the ammonium recovery rates in the samples, indicated in all the three layers in both
the groups. Exceptions to this are only in the presence of Blitox and Paraquat. Likewise, the values of nitrite and nitrate, which are much lower in the pesticide treated samples, in all the three layers, suggest that, the pesticides influence on nitrification activity. As before an exception to this, is only Paraquat.

Most probable number estimations revealed scanty populations of autotrophic nitrifiers present in the samples. No significant difference between the samples from group I and III can be registered by studying the five groups of organisms. It is evident, that the ureolytic strains play an important role in the soil microbiology. Since ureolytic strains have also been proved to contain nitrifying strains (Bezbarush 1983) and the results recorded here have indicated heterotrophic nitrification, it is obvious, that the nitrification activity in these soils, is chiefly due to heterotrophic nitrification.

Judging from the population density of nitrogen fixing types it is clear, that nitrogen fixation also occurs in tea soils despite the acid pH. The low population density of the denitrifying types in the samples from group IV suggest that the denitrifier population may have increased only in the soils under tea cultivation.
Whether this is due to the constant use of pesticides and chemical fertilizers it can not be definitely stated from the results of the present investigations.

Free living nitrogen fixing strains appeared chiefly to belong to the genus *Diazia*. The most interesting feature here appears to be the number of strains of *Arthrobacter* which carryout the function of nitrogen fixation. From the pH optima studies it is indicated that all the strains function efficiently in an acid environment, pesticide tolerance and response to nitrogen fixation activity appears to vary from strain to strain. This suggests that the strain response to the pesticide, is also important in determining the extent of the influence of a given pesticide in the environment. Since none of the strains grew, either in the presence of Dalapon, or Paraquat, it can be said that these two pesticides may reduce nitrogen fixing activity in the soil environment. It can be recalled from fig. 8 and 9 that soil samples treated with Dalapon showed slightly higher values of ammonium than the controls. Paraquat on the otherhand showed considerably higher values than those of the controls particularly when the concentration of Paraquat was more. While this data in case of Paraquat can be explained as degradations in the Paraquat molecule and the subsequent reduction of the nitrogen from Paraquat, in case of Dalapon the increase in the ammonium values could only have come about through the death and lysis of organisms.
susceptible to Dalapon. Since several strains showed enhanced nitrification activity (Table 11) in the presence of 2,4-D, Kelthane, Tedion, Thiodan and Blitox it is unlikely that these pesticides negatively influence nitrogen fixation. It is interesting that, although only 11 strains survived in the presence of Blitox, there was an enormous increase in the ammonium values in the cultures containing Blitox. Likewise, enhanced nitrogen fixing activity in the presence of either 2,4-D or Kelthane or Tedion or Thiodan also indicates that the strains responded to the pesticides determines the rate of nitrogen fixation. Thus the population density of the strains responding to the different pesticides, can either positively or adversely effect nitrogen fixation in the presence of pesticides. Strains such as *Dermia* 102, 113, 140, *Arthrobacter* 111, 114, 135 and 146 which tolerate several pesticides and also enhance nitrogen fixing activity, in the presence of the pesticides may eliminate any inhibition to nitrogen fixation, caused by these pesticides. Likewise, the 7 Blitox tolerating strains which showed enormous increase in the presence of Blitox may over shadow the inhibition caused by Blitox to other nitrogen fixing strains. Thus, although the enhanced ammonium values in the soil samples (Fig. 6) in the presence of Blitox can be taken as enhancement due to death and lysis of the cells as in case of Dalapon,
the behaviour of the isolated strains in the presence of Blitox suggest that Blitox may indeed enhance nitrogen fixation in soils. This cannot be conclusively proved here unless population density of individual strain in this soil environment can be ascertained.

Among the denitrifying strains isolated, only 5 strains of Bacillus namely B. cereus, B. subtilis, B. pumilus, B. lentus and B. megaterium tolerate all the pesticides tried. Since almost all the strains tolerated Dalapon and 2,4-D and several strains Kelthane, the influence of these pesticides on denitrification activity is once again suggested. It can be recalled from fig. 16, 17 that high nitrite values in nitrate amended samples, low nitrate values in nitrate amended samples (fig. 12 and 13) and high rate of N-volatilization in nitrate amended samples (fig. 20 and 21) also showed that 2,4-D and Dalapon were active in denitrification. The enhanced denitrification activity observed in the presence of these two pesticides in pure cultures (Table 12) confirms the view that active denitrification takes place in the presence of these two pesticides. Likewise, the enhanced nitrite and volatilized-N and reduced nitrate in the samples amended with Kelthane (fig. 16-23) and the enhanced denitrification activity observed in pure culture of several strains also confirms the positive influence of Kelthane in denitrification. In the presence of all the other pesticides, denitrification appears to be limited to the 5 strains of
Bacilli tolerating them.

Since none of the strains of autotrophic nitri- 
flora isolated, grew in the presence of any of the pes- 
ticide triad, it is unlikely, that the nitrification 
observed in the soil samples in the presence of pesticides, 
is due to the activity of these nitrifiers. As 
active nitrification occurred, particularly in the presence 
of Paraquat and also in the presence of Blitox and other 
pesticides, nitrification has clearly been brought about, 
by strains not elaborated here.

The influence of pesticides Dalapon, Paraquat, 
2,4-D, Blitox, Kelthane, Tedion and Thiodan on the nitro- 
gen fixation activity measured in terms of Nitrogen accu- 
mulated in Nitrogen free media is depicted in Table 10. 
From this Table 10 it is clearly indicates that none of 
the strains either *Pseudomonas* or *Bacillus* described in 
Table 9 survive in the presence of any of the pesticides. 
Similarly, Dalapon and Paraquat were lethal to all the 
nitrogen fixing isolates. Ammonium, nitrite and nitrate 
values, estimated in the 7 day old culture, in the nitro- 
gen free media, as depicted in table 10 indicated the 
difference and the ability of the strains to fix-nitrogen. 
It is noted that the *Derxia* spp (102) yielded more nitrogen 
in the presence of Blitox, Kelthane, Tedion and Thiodan, 
*Derxia* (102) yielded more in the presence of Kelthane, 
*Derxia* (113) in the presence of 2,4-D and Thiodan, *Derxia* 
(113) in the presence of Kelthane, *Derxia* (116) in the pre- 
sence of 2,4-D and *Derxia* (121, 132 and 140) in the presence 
of Blitox (Table 10).