# FIXATION OF NITROGEN BY RICE SOILS AND RICE PLANTS.

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## 1. *Introduction.*

DURING the study of the nitrogen recuperation of soils, which the author is carrying on for some years past, it was observed that generally during the growing period of a crop the cropped plot showed less nitrogen than the uncropped plot but with rice lands the reverse was the case. In one instance where three crops were taken in one year it was observed that during the time the rice was growing the cropped plot had more nitrogen than the uncropped plot but as soon as other crops like Cabbage, etc., and Sweet potato came in, the cropped plot showed less nitrogen than the uncropped plot during cropping period. This naturally created a suspicion as to whether rice crop helped in some way to fix nitrogen in the soil.

It is also a well-known fact that in most of the rice lands in India sufficient nitrogen is always present to supply about 30 lbs. of nitrogen per acre annually to the crop grown on them. It seems, there is a natural fixation of nitrogen in these soils to compensate the loss due to (1) removal of nitrogen by rice crop, (2) removal of nitrogen by washing due to heavy rainfall, and (3) the loss due to decomposition of nitrates in the water-logged condition.

J. Sen (1924 and 1930) says, " An examination of the contents of roots of rice plants conducted by Dr. Harrison at Coimbatore had yielded indications of the presence of bacteria apparently capable of assimilating free nitrogen. " He further says, " The development of bacterial growth in the solutions, which contained no nitrogen, clearly indicated an assimilation of nitrogen from the gaseous atmosphere.

" This demonstration of an occurrence of symbiotic nitrogen fixing organisms within the roots of the rice plant throws new light on the problem of nitrogen nutrition of rice and opens out many interesting lines of study. "

B. Viswa Nath (1932) says, " An interesting observation arising from the work in Coimbatore laboratories is that there are indications that paddy plant is capable of fixing atmospheric nitrogen. This may be possible seeing that paddy lands which have not been manured for years still maintain the yield, but the suggestion requires confirmation."

In order to ascertain the truth of the results of the previous experiments, work was done along two lines. Plot experiments were done at Karjat and Ratnagiri and laboratory experiments were carried out at Poona.

## 2. *Field Experiments.*

In every place a suitable area was chosen and was divided into two equal plots. The plots received the same treatment except that one was cropped and the other was not.

Samples were taken by randomisation. From each plot 12 samples of surface soil of 6″ depth were taken and thoroughly mixed up and a composite sample was taken, dried at 60° C. to prevent changes and then brought to Poona. Soil samples for moisture were taken separately. The randomised spots from which the samples were taken, were distributed fairly evenly over the whole plot.

The methods of analysis were the same as those followed in the previous publications on nitrogen recuperation of soils.

*Special precautions taken in analysing the samples.*—

(*a*) Often investigators take 5 to 10 grammes of soil sample for nitrogen determination. 20 grammes of soil were taken to minimise errors in final figures which increase by multiplication.

(*b*) 0·05 N sulphuric acid was used for total nitrogen.

(*c*) Blank determinations were done and corrections made accordingly.

(*d*) Glass distilled water was used all through.

All these precautions were taken to minimise the working error as much as possible.

*Working error.*—Whatever the precautions one may take there is sure to be some working error in the case of soil nitrogen which is present in small quantities. It is essential that working error is determined to get an idea as to what extent there is real fluctuation after making necessary allowance for all sorts of errors. Soon after the work was started a soil sample was collected by randomisation of the spots, etc., on a plot. This composite sample was divided into 8 parts and soil from each of these was analysed for total nitrogen. The figures obtained were as follows :—

*Milligrams of Nitrogen in* 100 *Grammes of Oven Dry Soil.*

| Serial No. | Total nitrogen |
|---|---|
| | Milligrams. |
| 1 | 77·52 |
| 2 | 77·52 |
| 3 | 78·38 |
| 4 | 77·98 |
| 5 | 77·75 |
| 6 | 77·75 |
| 7 | 78·38 |
| 8 | 78·18 |

The standard deviation for total nitrogen comes to 0·3527. Any difference which is more than three times the standard deviation, *i.e.*, 0·3527 × 3 = 1·0581 is significant.

1. *Experiments at Karjat.*—At Karjat on Government Experimental Farm two sets of experiments were done—one in the cold season of 1934–35 and the other in the rainy season of 1935.

(*a*) For winter season crop of rice, an area was divided into two equal plots adjacent to each other measuring 33′ × 33′ each. On one plot transplanted rice was grown and the other was left without a crop. This plot, however, received water and all other treatments just the same as the cropped plot. No plot received any manure. Ploughing and cross ploughing was given on the 10th of December. Puddling was done on the 15th and the transplanting of rice seedling was done on cropped plot on the 24th of December. The crop was harvested on the 8th of April. The plots were watered every day. The samples of soils were taken every month by randomisation and by preparing a composite sample from 12 spots from each plot. Temperatures and moistures for both the plots were nearly the same. The difference in temperatures never went beyond one degree Centigrade and the difference in moistures never beyond one per cent.

The following table gives the nitrogen month to month from the cropped and uncropped soils.

TABLE I.

*Soil moistures in per cent. and total nitrogen in milligrams per 100 Grammes of oven dry fine soil.*

| Time | Cropped | | Uncropped | |
|---|---|---|---|---|
| | Soil moisture | Total nitrogen | Soil moisture | Total Nitrogen |
| | Per cent. | Mgms. | Per cent. | Mgms. |
| Last week of December, 1934 | 32·0 | 78·02 | 30·87 | 78·84 |
| January, 1935 | 35·71 | 83·80 | 31·02 | 80·53 |
| February | 28·0 | 76·89 | 27·83 | 73·53 |
| March | 21·78 | 75·19 | 22·5 | 77·83 |
| April | 4·45 | 75·09 | 4·5 | 74·64 |

(*b*) The rainy season plots were of the same size as those for the winter season and were adjacent to each other. Both of them were treated exactly alike the only difference being one carried a rice crop and the other was without one. The plots received ploughing on the 21st of June, cross ploughing on the 28th June, puddling on the 9th of July and transplanting on the 11th of July. One weeding was given on the 8th of August and another on the 19th of September. The crop was harvested on the 10th of November.

Soil samples were taken from month to month by randomisation from both the plots as in the other experiment. The variations in total nitrogen in the two plots are given in Table II below.

2. *Experiments at Ratnagiri.*—At Ratnagiri a field experiment was tried on a winter season crop of 1934-35. Two adjacent plots of one *guntha* each were taken and ploughed on the 12th of January, 1935. The plot was watered and sowing was done by broadcasting germinated seed on one plot on the 16th of January. Both the plots were watered every morning. The cropped plot was harvested on the 16th of April.

Samples of soils were taken by randomisation from both the cropped and uncropped plots. The variations in total nitrogen in the two plots are given in Table III below.

TABLE II.

*Soil moistures in per cent. and total nitrogen in milligrams per 100 grammes of oven dry fine soil.*

| Time | Cropped | | Uncropped | |
|---|---|---|---|---|
| | Soil moisture | Total nitrogen | Soil moisture | Total nitrogen |
| 1935 | Per cent. | Mgms. | Per cent. | Mgms. |
| Last week of July | 40·22 | 85·53 | 40·22 | 87·86 |
| August | 40·22 | 85·15 | 40·22 | 82·45 |
| September | 39·85 | 88·49 | 40·07 | 85·30 |
| October | 33·45 | 85·80 | 32·57 | 83·05 |
| November | 32·64 | 84·49 | 30·79 | 80·71 |

TABLE III.

*Soil moisture in per cent. and total nitrogen in milligrams per 100 grammes of oven dry fine soil.*

| Time | Cropped | | Uncropped | |
|---|---|---|---|---|
| | Soil Moisture | Total Nitrogen | Soil Moisture | Total Nitrogen |
| | Per cent. | Mgms. | Per cent. | Mgms. |
| Second week of January, 1935 | 2·63 | 216·65 | 2·51 | 221·00 |
| February | 35·65 | 225·89 | 35·61 | 217·87 |
| March | 38·79 | 234·65 | 36·28 | 231·14 |
| April | 33·69 | 240·18 | 26·66 | 230·20 |
| May | 2·96 | 223·42 | 2·63 | 205·11 |

The figures for total nitrogen given in Tables I, II and III show that during the growing period of the rice crop, the cropped plot shows more nitrogen than the uncropped plot. These results confirm those obtained in the experiments at Belgaum and Karjat given in Part IV of Nitrogen recuperation of soils mentioned in the beginning of this paper. (Sahasrabuddhe and Abhyankar, 1936.)

In all five field experiments were tried :—

(1) At Belgaum in the rainy season of 1933.
(2) At Karjat in the rainy season of 1933.
(3) At Karjat in the winter season of 1934-35.
(4) At Ratnagiri in the winter season of 1934-35.
(5) At Karjat in the rainy season of 1935.

The first two experiments are given in Part IV of nitrogen recuperation. These five experiments include three varieties of rice soils and also include rainy and winter seasons. In all the trials, total nitrogen in the cropped plots tends to be higher than that in the uncropped plots. This happens notwithstanding the fact that the rice crop, when growing, draws upon the nitrogen of the soil to build its own tissues. This is perhaps, why the rice lands have been able to maintain their fertility, although at a low level, without manuring for centuries together. Many of the rice soils have more nitrogen than other soils in the Bombay Presidency although rice soils are likely to lose more nitrogen than other soils, due to the washing action of heavy rainfall and due to the decomposition of nitrates in water-logged conditions. The maintenance of the nitrogen level of rice lands seems therefore to be due to the nitrogen fixing power of the rice soils, especially in the presence of the growing rice crop.

### 3. *Laboratory Experiments.

Laboratory experiments were carried out to ascertain whether a sterilised nearly nitrogen-free solution, like that of Ashby's can fix atmospheric nitrogen (1) when rice soil is introduced in it, and (2) when rice seedlings are grown in the solution either with or without rice soil.

1. *First series.*—The first series of experiments was started on the 6th of January, 1935, and closed after ten days on the 16th of January. Nitrogens of all the contents of the flasks excepting the plants, were determined. The bacterial and other actions in the flasks were stopped on one and the same day by adding sulphuric acid to the flasks :

The results obtained are given below.

* This was submitted as a paper to the International Congress of Soil Science, held at Oxford (England) in August, 1935.

TABLE IV.

*Total nitrogen in milligrams at the end of ten days in each flask.*

| Treatment | Nitrogen to begin with | Nitrogen after 10 days | | | Average of the three |
|---|---|---|---|---|---|
| | | I | II | III | |
| 1. Ashby solution .. .. | 0·66 | 0·66 | 0·66 | 0·67 | 0·66 |
| 2. Ashby solution and rice root extract* .. .. .. | | 0·76 | 0·84 | 0·87 | 0·82 |
| 3. Ashby solution and rice root extract and rice soil (one gram) .. .. | | 5·24 | 6·29 | 6·94 | 6·16 |
| 4. Ashby solution and rice root extract and rice soil (one gram) and rice plants growing .. .. .. | | 11·46 | 12·86 | 12·98 | 12·43 |

* Roots were crushed with a small quantity of water and 5 c.c. were used.

The soil contained 0·077 per cent. of nitrogen, *i.e.*, in one gramme there was 0·77 milligram of nitrogen. The figures show that Ashby's solution by itself does not fix any nitrogen. Addition of rice root extract does not help in fixing nitrogen (the slight increase seen is perhaps due to the nitrogen in the root extract itself). Addition of rice soil decidedly adds nitrogen and the presence of the growing rice roots further increases the nitrogen contents. There is something in the rice soil which has the power of fixing nitrogen. This is further enhanced by the growing roots of the rice plants. The results of the three lots all point to the same conclusions.

2. *Second series.*—A second series was started on the 2nd of February and was closed after 10 days. To begin with all the flasks had 150 c.c. of Ashby's solution but as the flasks in which rice plants were growing showed less of liquid, 60 c.c. of Ashby's solution diluted was added to each of the flasks to have the same quantity of Ashby's solution in each. This series included the experiments done in two lots as given in the following table. The second lot had more plants than the first.

Excepting the first two experiments the duplicates do not agree with each other because the plants introduced were more in the second lot than in the first. But the trend of results is the same in both the lots. Flasks in experiment two show more nitrogen than those in experiment one because

TABLE V.

*Figures for total nitrogen for the two lots are given in milligrams for each flask.*

| Treatment | Total nitrogen in each flask after 10 days | |
|---|---|---|
| | I | II |
| | Milligrams | Milligrams |
| 1. Ashby solution only .. .. .. | 1·03 | 1·02 |
| 2. Ashby solution and one gramme sterilised rice soil .. .. .. | 1·82 | 1·82 |
| 3. Ashby solution with plants growing in solution .. .. .. .. | 3·6 | 6·77 |
| 4. Ashby solution and one gramme air dry rice soil .. .. .. .. | 9·78 | 10·59 |
| 5. Ashby solution and one gramme sterilised rice soil with plants growing in solution | 10·22 | 13·26 |
| 6. Ashby solution and one gramme air dry soil with plants growing in solution .. | 12·02 | 15·76 |

the soil added in the second experiment contains nitrogen which is equal to the difference in nitrogen in one and two. This shows that sterilised rice soil has no power of fixing nitrogen.

When rice plants grow in culture solution there is definite fixation of nitrogen. This is perhaps because the roots carry nitrogen fixing inoculum as the plant roots in the experiment were washed but not sterilised.

Air-dry soil introduced in the fourth experiment supplies nitrogen fixing inoculum and also a suitable medium in the form of soil. It is hence, perhaps, that good fixation of nitrogen is observed. In Experiment 5 the inoculum is introduced by the rice plant roots and a suitable medium is supplied by the sterilised soil.

In the sixth experiment there is the inoculum introduced by the plant roots as also by the air-dry soil and the medium with rice soil is quite suitable for nitrogen increase and hence the highest figures of nitrogen are given by the sixth experiment.

3. *Third series.*—In the experiments conducted so far, the rice plants were brought from rice fields at Karjat. That the Karjat soil contains the

inoculum is clearly proved by the experiments. It is however not clear whether any inoculum is carried within the seed. In order to find this out, seedlings were prepared under sterilised conditions from rice seed washed to sterilise the surface and these seedlings were then allowed to grow in sterilised Ashby solution as in the previous experiments. The Ashby solution used in this case, was reduced in strength by diluting it with three times its volume of sterilised water. This was done to give a dilute solution to very tender seedlings.

After a period of 10 days from the time the seedlings were introduced in the culture flasks, the nitrogen fixed as determined in each case is given below.

TABLE VI.

*Milligrams of total nitrogen in each flask after ten days.*

| Treatment | Total nitrogen in each flask | |
|---|---|---|
| | I | II |
| | Milligrams | Milligrams |
| 1. Ashby solution only .. .. .. | 0·81 | 0·81 |
| 2. Ashby solution with plants from sterilised seed .. .. .. .. .. | 1·05 | 0·96 |
| 3. Ashby solution and one gramme sterilised rice soil with plants from sterilised seed .. | 1·88 | 1·70 |
| 4. Ashby solution and one gramme air-dry rice soil with plants from sterilised seed .. | 3·59 | 3·41 |

The results of the duplicates agree with each other. The slight excess of nitrogen of the second experiment over that of the first must be due to small quantity of root material dropped in the culture during washing, etc. The excess of nitrogen in three over that in two is due to the nitrogen in the sterilised soil. It therefore clearly shows that no nitrogen fixing inoculum is carried within the rice seed. The fourth experiment shows definite fixation of nitrogen and this must be put down as due to the inoculum in the air-dry rice soil.

It can, therefore, be safely stated that nitrogen fixing inoculum is not carried within the rice seed although it may be carried on the surface of the seed. On the other hand it is sure that the inoculum is in the rice soil and may be carried along with the soil. The presence of growing rice roots helps this inoculum in fixing larger quantities of nitrogen than the soil would otherwise fix because the rice roots form a good host.

### *4. Conclusion.*

It was observed in the previous experiments done in connection with the nitrogen recuperation of soils that the cropped rice plot shows more nitrogen than the uncropped rice plot during the growing period of the rice crop. These results have been confirmed by the field experiments, in the winter season at Karjat and Ratnagiri and in the rainy season at Karjat.

These results received further confirmation by the experiments done in the laboratory which show that the rice soils have the power of fixing nitrogen and this fixation is helped by the presence of the growing roots of the rice plant. It has been shown that the rice seed does not carry within it any nitrogen fixing organisms.

### REFERENCES.

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