A NOTE ON THE NITROGEN SUPPLY OF RICE SOILS.

By P. K. De, *Agricultural Chemistry Section, Dacca University.*

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It is the experience of every cultivator of Bengal that, even in absence of any manure, rice can grow in the same field year after year, without causing any depletion in the fertility of the soils. Howard (1924) stated that in Bengal and in Burma large rice crops have been grown on the same land year after year for centuries, yet no change in the fertility of the soils has been observed. So far as the supplies of nutrients like phosphate, potash, etc. are concerned, crops may find them in the soils in amounts to meet their requirements over long periods. But as regards nitrogen, the supply of this element in the soil is relatively poor specially in Indian soils. It, therefore, becomes a matter of surprise how rice could get the large supply of nitrogen that was required for its growth over centuries. It suggests itself that atmospheric nitrogen is rendered available to the plant by some natural process. This can be done in two different ways : nitrogen may be fixed in the soils by some non-symbiotic processes and subsequently made available to the plants, or the plants themselves may assimilate atmospheric nitrogen, like legumes. At present we do not know of any plant other than legumes, which has been definitely shown to possess the power of assimilating elementary nitrogen, though several workers have claimed to have been able to grow some non-legumes in absence of nitrogen. Rice never suffers from nitrogen deficiency, and the possibility of the fixation of nitrogen by this crop deserves very careful examination. In this connection it is noteworthy that Sen (1929) has demonstrated the occurrence of a nitrogen-fixing organism within the rice root, while Viswanath (1932) obtained indications that rice may assimilate atmospheric nitrogen.

A detailed investigation on the nitrogen supply of rice was started in this laboratory in 1931. It is not possible to give within the scope of this note a complete account of the results obtained so far and the details of experiments or of the references relating to different subjects. A brief account of the more important results and the conclusions they led to has been given in this note.

Fixation of nitrogen in the rice soils under water-logged conditions.[1]

The abundant growth of algæ in the submerged rice soils and the claims that certain algæ are capable of fixing atmospheric nitrogen, either independently or working in symbiosis with the nitrogen-fixing bacteria, suggest that some fixation may take place during the period the soil remains water-logged.

[1] A paper embodying the results of this investigation has been communicated for publication in the *Indian Journal of Agricultural Science*.

As a result of extensive experiments, it is now definitely known that nitrogen is fixed in the rice soils during the period of water-logging. The results in Table 1 show that soils slightly alkaline in reaction fix much more nitrogen than those with lower P_H. In the former soils, abundant growth of algæ took place but in the latter the growth was much poorer. The addition of calcium carbonate to the latter soils, however, stimulated both algal growth and nitrogen-fixation (Table 2).

TABLE 1.

Fixation of nitrogen under water-logged conditions.

P_H of the soil.	N fixed, as p.p.m.
8·2	333
8·4	330
8·1	275
7·7	112
7·6	98
5·1	79
4·9	41
4·9	11

TABLE 2.

Effect of $CaCO_3$ *on the fixation of nitrogen.*

	N fixed, as p.p.m.	
P_H	Without lime.	With lime.
4·9	41	116
4·9	11	142
6·2	149	205
5·1	79	138

It has been shown that the fixation of nitrogen in the water-logged soils is not a bacterial process but an algal one. But the fact whether algæ themselves fix nitrogen or whether they do so in symbiosis with other organisms remains undetermined for the present. The following species of algæ were identified by Professor F. E. Fritsch, F.R.S., from the nitrogen-fixing cultures isolated from Faridpur soil :—*Plectonema notatum* Schmidle, *Phormidium orientale, Anabœna sphœrica* and an undeterminable green unicellular alga.

Fixation of nitrogen in the rice soils under dry conditions.

During the few winter months, the condition of the soils both as regards temperature and moisture are eminently suitable for the growth of *Azotobacter* and other aerobic nitrogen-fixing bacteria. Some fixation of nitrogen may, therefore, take place during this period. Experiments, however, showed that although fixation of nitrogen took place in some of the soils, yet by no means can it be regarded to be as general as the fixation under water-logged conditions. There were even losses of nitrogen from some soils, specially when they were treated with straw. The most interesting result in this connection is that considerable fixation was observed in some soils which did not contain *Azotobacter*. This led to a search for new nitrogen-fixing organisms in the rice soils and as a result four different species of organisms (probably belonging to the genus *Azotobacter*) were isolated in pure conditions. These organisms were characterised by their slow growth in the nitrogen free mannite medium, large slime formation, non-motility and the absence of pigment formation.

Fixation of nitrogen by the rice plant.

Rice plants were grown on purified sand in glazed pots watered with a nitrogen-free nutrient solution. Though the plants were never so healthy as in the fields, yet they grew up to maturity with tillers and grain formation. A carefully prepared nitrogen balance-sheet showed considerable increase of this element (Table 3). The experiment was repeated in the following year and the previous observation confirmed (Table 4) :—

TABLE 3.

Sand culture experiments on the growth of rice plants in absence of combined nitrogen. (1933).

N as mgm.

Pot	Treatment	Total N at start in sand and in seeds sown	Residual N in sand after the experiment	Nitrogen in the crop			Total nitrogen recovered	Grain
				Straw	Root	Grain		
1	Uncropped	118·5	120·4	...	...	...	120·4	1·9
2	Cropped	119·8	139·5	12	7·6	20·4	179·5	59·7
3	,,	119·8	114·6	16·9	11·3	17·4	160·2	33·7
4	,,	119·8	116·8	20·2	10·8	10·3	158·1	31.3
				TABLE 4. (1934).				
1	Uncropped	114·6	118·5	...	...	...	118·5	3·9
2	,,	114·6	119·6	...	...	...	119·6	4·3
3	Cropped	116·3	107·0	24·8	8·7	15·5	156·0	39·7
4	,,	116·3	110·8	22·7	6·9	15·0	155·4	39·1
5	,,	116·3	105·1	16·9	7·0	14·1	143·1	26·1
6	,,	129·8*	109·9	18·4	5·6	10·8	144·7	14·9
7	,,	129·8*	114·6	44·8	12·5	23·7	195·6	65·8

In both sets of experiments, the nitrogen in the sand after the experiment remained practically unchanged. This indicated that the increase is due to the plants themselves. To ascertain whether the plants can assimilate elementary nitrogen without the help of other organisms, sterilised seeds were sown over pure quartz sand in wide test tubes, watered with sterile nitrogen-free nutrient solution, and constantly aerated with air passing through a number of sterile cotton plugs. The plants did not grow well under these conditions and their nitrogen contents were not significantly greater than those added with

* Treated with 13·5 mgm. of N added as KNO_3.

seeds. This suggests the inability on the part of the rice plant to assimilate nitrogen directly. In an attempt to discover whether there is a symbiotic relation between rice plants and any other nitrogen-fixing organism, it was observed that one or more nitrogen-fixing organisms exist within rice leaves. Small pieces of rice leaves were inoculated into a nitrogen-free cane sugar solution containing the necessary minerals. Growth took place and the determination of nitrogen after 3 weeks showed an average fixation of 2 mg. of nitrogen. A large number of leaves were examined in different periods of growth, in different seasons and from different localities. Fixation took place in every case. This shows that these organisms are the normal inhabitants of rice leaves and not contaminants or occasionals. Growth was observed also on silica plate inpregnated with cane sugar mineral solution, when inoculated with small pieces of rice leaves.

TABLE 5.

Mgm. of nitrogen fixed in 100 *c.c. of the medium when inoculated with weak growths of 'leaf' bacteria.*

1	·98	
2	·84	
3	·98	Mean 1·12 mg.
4	1·26	
5	1·4	

Loss of nitrogen from water-logged soils.

Investigation on the loss of nitrogen from water-logged soils showed that from unmanured soils little nitrogen is lost in the drainage, but from the soils treated with different organic manures the loss is considerable. Nitrogen is lost from the manured soils in the form of ammonia, non-amino nitrogen compound and humin nitrogen (nitrogen precipitated with MgO). Though a certain amount of nitrogen is lost in the gaseous form, particularly from soils of low nitrifying capacity, it is very small in comparison with the heavy loss brought about by bio-chemical processes. As early as 1913, Harrison and Aiyar demonstrated the evolution of elementary nitrogen from water-logged soils. No explanation has been given as to how elementary nitrogen can be evolved as a result of the decomposition of soil organic matter. The present investigation showed that this loss is not due to the decomposition of ammonium nitrite (Table 6). But it has been found that the loss takes place when the oxidation of ammonia begins. Evidences have been obtained in support of the following reaction :—

In normal dry soils the oxidation of ammonia takes place in the following way—(Corbet, *Biochem. J.*, 1935) :—

$$2NH_3+O_2=2NH_2OH\ldots\ldots\ldots\ldots\ldots(1)$$
$$NH_2OH+O_2=N(OH)_3\ldots\ldots\ldots\ldots\ldots(2)$$
$$N(OH)_3=HNO_2+H_2O\ldots\ldots\ldots\ldots\ldots$$

In water-logged soils, the supply of oxygen is much limited. The reaction (2) therefore takes place in the following way :—

$$4NH_2OH+O_2=2N_2+6H_2O \dots\dots\dots\dots (3)$$

TABLE 6.

Decomposition of ammonium nitrite in water-logged soils.

Period of exposure to sun		Total N at start	N recovered as ammonia and nitrite	Total N recovered	Loss
24-5-36 to 13-6-36	..	19·35 (Quartz flask and sterilised).	6·3 *	18·48	·87
,, ,, ,,	..	19·35 (Jena flask and unsterilised).	*nil*	17·36	1·99
22-6-36 to 4-7-36	..	15·96 (Quartz flask).	5·32*	14·84	1·12
,, ,, ,,	..	15·96 (Jena flask).	3·92*	15·33	·63

In the first soil (P_H 4·9), the amount at start 19·35 mg. includes 5·49 mg. of N added as ammonium nitrite (sodium nitrite and ammonium chloride in equivalent amount).

In the second soil (P_H 8·1), 6·37 mg. of N were added as ammonium nitrite.

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* Includes a little organic nitrogen as the estimations were made by distilling soil with Devarda's alloy and magnesium oxide.