Studies on the Soils in Rice-field.

II. General Microbiological Investigation.*

By

Arao Itano and Satiyo Arakawa.

[October 13, 1928]

Introduction:

In a previous paper,[1] some of the ecological factors of rice-field soil were given with a special emphasis on the carbon-nitrogen ratio and hydrogen ion concentration. In this paper, the temperature and amount of rainfall were considered besides the other factors already considered and a general microbiological analysis was made as to the following items:

1. Quantitative determination of bacteria, actinomycetes and fungi.
2. The ammonifiers.
3. The nitrifiers.
4. The denitrifiers.
5. The nitrogen fixers.
6. The cellulose fermenters.
7. The evolution of carbon dioxide.

The importance attached to such an investigation is hardly over-emphasized as it has been noted by other investigators in America and Europe, and the numerous number of investigations have been undertaken. However their investigations have been largely confined to the soils in dry farm and not of the rice-field such as found in Japan. It is the purpose of this paper to give a general systematic survey of rice-field soil and hope to obtain some valuable information as to the soil population in the rice-field and their activities.

The report will be given in two parts, namely Part I. Ecological Factors and Part II. Microbiological Investigation with subdivisions for each.

---

1) A. ITANO and S. ARAKAWA, Berichte d. Ōhara Inst. etc. III, 321, 1927.

* In our previous publication in the series, was noted as 'Studies on the Carbon-Nitrogen Ratio and Microbiological Investigation of the Soil in Rice-field,' but it was changed to the present title since so many other factors had to be considered.

Experimental:

The soil sample for this investigation were taken from Plot II which is the normal control plot for our investigation and of which description was given in our previous investigation.[1]

It was necessary to develope and select the satisfactory method for the investigation and somewhat detail consideration will be given later under each heading.

Part I. Ecological Factors:

A. Temperature.

The factors such as the C : N ratio and pH values were given in our previous publication.[1] Since the temperature has very intimate relation to the bilogical activities, it was recorded as to the atmosphere, irrigation water, soil surface and in the surface soil. For a certain period, the field is flooded with the water and it was necessary to make the record of water. The records were taken three times daily at 8.00 A. M., noon and 4.00 P. M., but only the average for every ten days during the growing season of rice is givn here, in Table I.

Table I.

Temperature (°C) of Air, Irrigation Water, Soil Surface and Soil.

Date.	Air.	Irrigation water.	Soil surface.	Soil.
7/1—10	26.3	28.7	28.5	26.8
7/11—20	29.8	34.0	33.5	28.6
7/21—31	30.1	31.3	32.3	29.9
8/1—10	27.1	29.6	27.9	26.4
8/11—20	27.6	28.7	29.2	27.7
8/21—31	29.8	29.2	28.8	27.8
9/1—9	25.3	24.1	24.6	24.3
9/10—19	26.3	24.1	25.0	25.0
9/20—29	21.9	—	21.7	21.1
9/30—10/9	22.4	—	21.1	20.4
10/10—19	19.6	—	17.8	17.6
10/20—26	19.6	—	17.0	16.4
10/30—11/8	17.0	—	14.9	14.5
11/9—18	16.4	—	15.0	14.1
11/19—28	14.5		12.8	11.6

1) A. ITANO and S. ARAKAWA, Loc. cit.

Fig. I.

Temperature °C. of Air, Irrigation Water, Soil Surface and Soil.

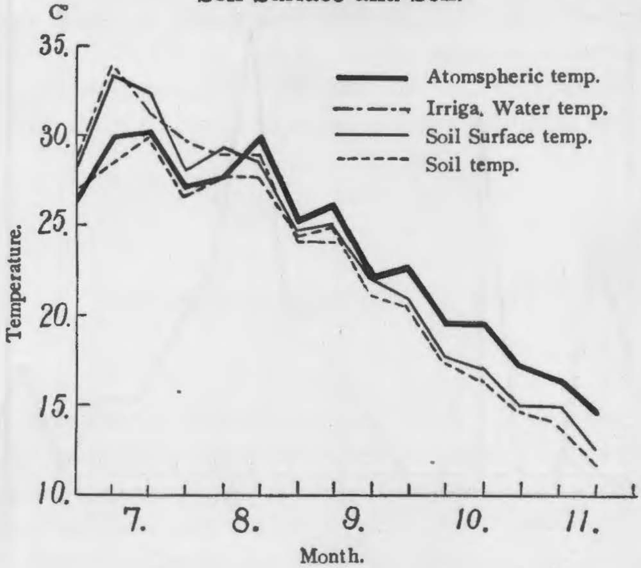


Table I. and Fig. I. indicate that the temperature of irrigation water and the soil surface reached as high as 34°C., which is 4 to 5 degrees higher than that of the air, and in the soil, from July 1 to August 20th. However, after August 21st, the atmospheric temperature gradually became higher than the others. This may be due to a fact that the sunlight is prevented by the grown up plants from penetration.

B. Rainfall.

The record of rainfall was obtained by the usual method at the weather bureau station at this Institute and is shown in Table II:

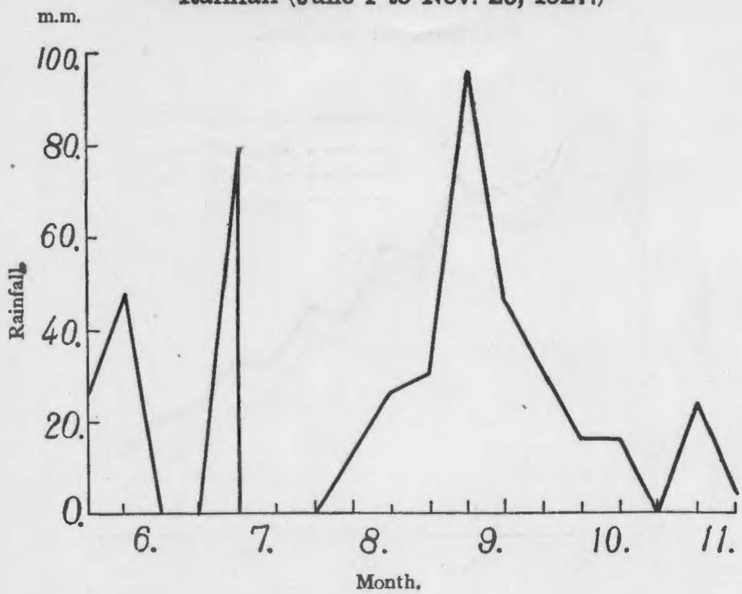
Table II.

Rainfall (June 1 to Nov. 20, 1927.)

Date.	Rainfall in mm.	Monthly total.	Date.	Rainfall in mm.	Monthly total.
6/1—10	23.6		9/1—10	94.8	
6/11—20	47.6	71.2	9/11—20	44.7	169.9
6/21—30	00.0		9/21—30	30.4	
7/1—10	77.5		10/1—10	16.4	
7/11—20	00.0	77.5	10/11—20	15.0	31.4
7/21—31	00.0		10/21—31	00.0	
8/1—10	12.5		11/1—10	23.8	
8/11—20	25.5	71.0	11/11—20	3.7	27.6
8/21—31	33.0				

Fig. II.

Rainfall (June 1 to Nov. 20, 1927.)



As it is indicated in Table II. and Fig. II, it was rather dry from the last part of June to the end of July, but a rapid increase as much as 170 mm. was reached in September and the rest of the season was normal.

C. *Moisture Contents of the Soil.*

The moisture contents of the soil in ricefield is naturally high since the field is usually located at the lower level than the irrigation water line, and for a certain season of the year, the field is kept flooded with the water. Table III. shows the moisture contents during the growing season of rice:

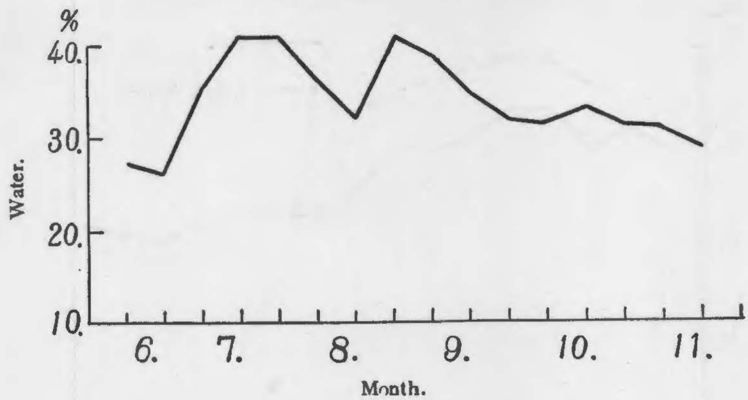
Table III.

Moisture Contents of of the Soil.

Date.	% Moisture.	Date.	% Moisture.
6/20	27.0	9/7	38.8
6/29	26.0	9/17	35.0
7/9	35.0	9/27	32.3
7/19	41.0	10/7	31.4
7/29	41.0	10/17	32.9
8/8	35.5	10/27	31.1
8/18	32.2	11/7	30.5
8/28	41.1	11/17	28.4

Fig. III.

Moisture Contents of the Soil.



As Table III. and Fig. III. indicate, the moisture contents of the soil was very high, from July 9 to August 28th while it had been flooded. After that, it became lower except when the rain came during September 8 to 17th.

D. Hydrogen Ion Concentration of Soil and Irrigation Water.

The hydrogen ion concentration in both the soil and irrigation water was determined by the quinhydrone method and the results are indicated in Table IV. and Fig. IV:

Table IV.

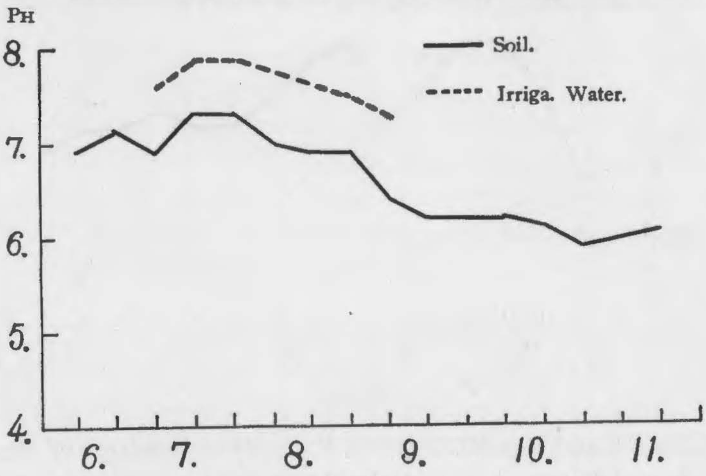
Hydrogen Ion Concentration of Soil and Irrigation Water.

Date.	pH		Date.	pH	
	Soil.	Water.*		Soil.	Water.
6/20	6.92	—	9/7	6.42	7.32
6/29	7.06	—	9/17	6.22	—
7/9	6.88	7.58	9/27	6.23	—
7/19	7.25	7.86	10/7	6.16	—
7/29	7.25	7.94	10/17	6.10	—
8/8	7.01	—	10/27	5.90	—
8/18	6.92	—	11/7	5.95	—
8/28	6.89	7.55	11/17	6.09	—

* Water denotes the irrigation water.

Fig. IV.

Hydrogen Ion Concentration of Soil and Irrigation Water.



The concentration of hydrogen ions in the soil varies during the season although the variation was very slight. Toward the end of season, it became slightly acid viz. PH 5.90. Recently KELLEY,[1] and BAVER[2] observed the similar phenomenon in connection with their investigation. That is toward the fall, the acidity in the soil increased by pH 1.0. On the other hand, the hydrogen ion concentration of the irrigation water remained alkaline during the season, reaching almost to pH 8.0 and the variation of pH value is in parallel with that of the soil. GILLESPIE[3] pointed out that with the water logging soil has tendency to resist the acidic change; also OSUGI[4] found that the pH value of irrigation water is greater than that of the soil. Very recently, SUBRAHMANYAN[5] in his comparative study of water logging soil, Indian and and Rothamsted soils, found that a rapid increase of pH took place at the begining and followed by the gradual decline and after fourty days, it increased again and reached higher than that of the initial period.

1) KELLEY, A. P., Soil Science, 16, 41, 1923.
2) BAVER, L. D., ibid, 23, 399, 1927.
3) GILLESPIE, L. J., ibid, 9, 199, 1920.
4) OSUGI, S., Nōgaku Kwaihō, No. 233, 115, 1922.

Part II. Microbiological Investigation.

A. Methods employed in the Investigation.

In such an investigation as this, the selection and developement of satisfactory methods are necessary, and as it is well recognized that the methods are still in course of developement by different investigators. In this investigation, the methods developed by WINOGRADSKY and WAKSMAN were employed as will be indicated by the reference under each problem.

I. Collection of Sample:

A sampling tube, 4 × 20 cm. brass tube with piston and handle at the head, was used. It was intended to collect the sample at ten centimeter depth since it is considered to contain the maximum number of organisms at that point by various workers, namely CHESTER,[1] BROWN,[2] KING and DORYLAND,[3] WAKSMAN,[4] and others.

The samples were collected at eight oclock in the morning under aseptic conditions as usual, taking three samples from each square in the plot so that twelve borings were made altogether. One or two centimeters of top soil was scraprd off by means of spatula and the boring was made, and a composite sample was prepared by mixing in a large porcelain dish. After a brass tube which is one size larger than the sampling tube was placed in a position, the water inside of the tube was sucked in a flask by releasing the stopcock. Then the soil sample was taken by means of the sampling tube from inside of the tube.

The soil samples were collected sixteen times during the season at ten days intervals, and five samples of the irrigation water were collected altogether.

II. Quantitative Determination of Bacteria and Fungi.

A. Quantitative determination of bacteria including actinomycetes:

The counting of bacteria was carried out by WAKSMAN's method,[5] by using the albumin agar. An average of ten plates is given in Table V. and Fig. V.

1) CHESTER, F. D., Del. Agr. Exp. Station, 13th Annual Report, 1902.
2) BROWN, P. E., Iowa Agr. Exp. Station, Bull. 8, 1912.
3) KING, W. E. and DORYLAND, C. J. T., Kansas Agr. Exp. Station, Bull. 161, 1909.
4) WAKSMAN, S. A., Jour. Bact. 1, 363, 1916.
5) WAKSMAN, S. A., Jour. Bact., 7, 339, 1921.

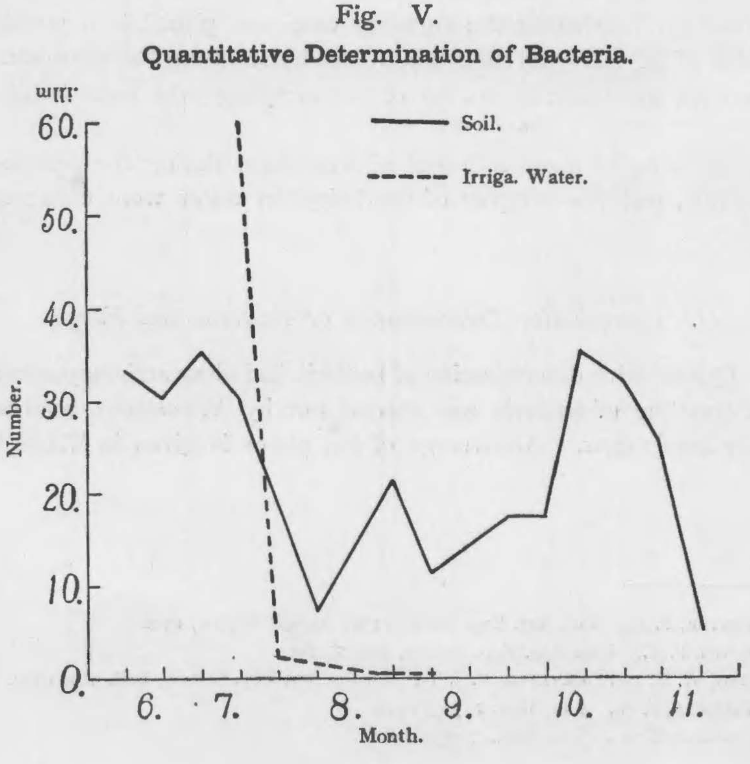
Table V.

Quantitative Determination of Bacteria.

Date.	Numbers in millions.		Weather condition.	Notes.
	Soil.	Water.		
6/20	33.21	—	fine.	
6/29	29.68	—	clear.	
7/9	35.11	568.46	"	manured & planted.
7/19	29.85	165.00	fine.	
7/29	19.17	0.60	"	
8/8	7.02	—	clear.	
8/18	13.66	—	"	
8/28	20.64	0.07	fine.	
9/7	10.74	0.03	clear.	
9/17	13.60	—	dull.	
9/27	16.77	—	"	
10/7	17.08	—	"	
10/17	34.59	—	clear.	
10/27	32.15	—	dull.	
11/7	25.40	—	clear.	harvested.
11/17	4.66	—	fine.	

Fig. V.

Quantitative Determination of Bacteria.



The results indicate that one gram of the soil contains 2151×10^4 on an average. A large number shown at the begining of the season is chiefly due to an application of fertilizers at that time. It decreased toward the begining of August and then kept on increasing until after the middle of October.

In America and Europe, the record from 200×10^4 to $20,000 \times 10^4$ per gram[1] are found. Since the number of organisms is influenced by many factors, no comparison can be made especially under entirely different conditions. Some found the maximum number in June and August[1] while BROWN[2] reported the maximum in Feburary and May; CUTLER[3], June and November as the maximum period.

Much less number of bacteria were found in the irrigation water than in the soil. On July 9th, more than $56, 846 \times 10^4$ was found. This however is due to the introduction of fertilizers at that time. Since all the determinations were carried out under the aerobic condition only, nothing can be said as to the extent of anaerobic organisms.

B. Quantitative determination of fungi:

The WAKSMAN's method[4] was used by making 1—1000 dilution and the plates were incubated for three days. An average of six plates is given in Table VI. and Fig. VI.

Table VI.

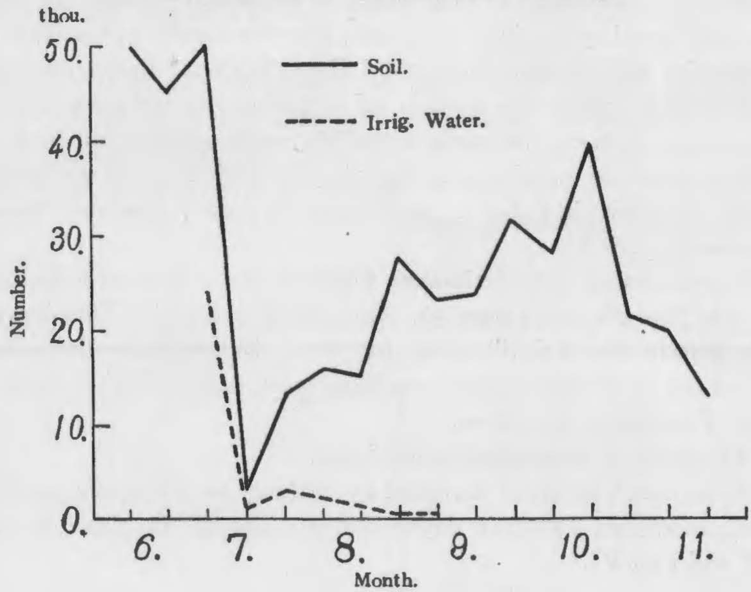
Quantitative Determination of Fungi.

Date.	Number in thousand.		Date.	Number in thousand.	
	Soil.	Water.		Soil.	Water.
6/20	50.9	—	9/7	23.1	0.2
6/29	45.5	—	9/17	24.2	—
7/9	49.2	24.4	9/27	32.5	—
7/19	3.1	0.5	10/7	28.5	—
7/29	13.1	2.6	10/17	40.0	—
8/8	16.3	—	10/27	21.8	—
8/18	15.2	—	11/7	20.9	—
8/28	28.4	0.2	11/17	13.7	—

1) WAKSMAN, S. A., Princ. of Soil Microbiology, 28, 1927.
2) BROWN, P. E. and HALVERSEN, W. V., Iowa Agr. Exp. Station Res. Bull., 56, 251, 1919.
3) CUTLER, D. W. & others, Phil. Trans. Roy. Soc. London, B, 21, 317, 1922.
4) WAKSMAN, S. A., Jour. Bact., 7. 339, 1921.

Fig. VI.

Quantitative Determination of Fungi.



Under the ricefield condition, it was expected to find abundant fungi present but in general from 1.3×10^4 to 5×10^4 were found which are less than those found by WAKSMAN[1], from 3×10^4 to 90×10^4 per gram; BROWN and HALVERSEN[2] reported from 4.2×10^4 to 13.1×10^4. This difference may largely be due to a fact that the reaction of soil and irrigation water was alkaline which favors the growth of bacteria and suppress the fungi as it was pointed out by WAKSMAN[3]. However the nature of curve is very similar to that of bacteria during the season.

III. Determination of Microbiological Activities.

The following tests were carried out besides the foregoing investigations:

1. Ammonification.
2. Nitrification.
3. Denitrification.
4. Nitrogen fixation.
5. Cellulose fermentation.
6. Carbon-dioxide production.

Each investigation will be described separately below:

---

1) WAKSMAN, S. A., Soil Science 2, 103; 3, 565, 1916—1917.

2) BROWN, P. E. and HALVERSEN, W. V., Iowa Agr. Exp. Station, Res. Bull., 56, 251, 1919.

3) WAKSMAN, S. A., Jon. Bact., 7. 339. 1921.

1. Ammonification.

The ammonifying power of the soil was determined by WAKSMAN's method.[1]

One percent peptone solution was inoculated with 1 cc. of 10% suspension of the soil or equivalent to 0.1 gm. of soil which was shaken for five minutes; incubated for four days at 28°C. The ammonia was determined by distilling with an addition of 1 gm. of magnesium oxide as recommended by HARPER[2]. The irrigation water was treated similarly.

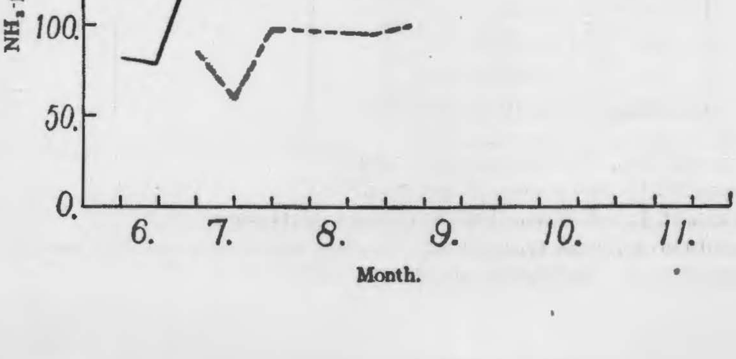
Table VII.

Ammonification Tests.

Date.	mg. ammonia.		Date.	mg. ammonia.	
	Soil.	Water.		Soil.	Water.
6/20	81.0	—	9/7	185.7	99.2
6/29	78.6	—	9/17	162.7	—
7/9	143.0	88.9	9/27	160.0	—
7/19	159.0	61.1	10/7	159.8	—
7/29	130.7	99.4	10/17	137.4	—
8/8	165.5	—	10/27	139.2	—
8/18	155.5	—	11/7	144.4	—
8/28	176.5	95.3	11/17	145.0	—

Fig. VII.

Ammonification Tests.



On July 9th, the ammonification became very marked and especially so at the end of August and the first part of September. The activity was kept up until the middle of November. The irrigation water however showed less activity through out the season.

Although it is an established fact[1] that the ammonification does not indicate the soil fertility, it is further investigated here among the different plots to see if it holds true in case of the ricefield.

It is interesting to note that RUSSELL and others[3] found that in case of the water logged soil, the ammonification process is brought about by the enzyme which was later substantiated by SUBRAHMANYAN[4] as diamidase. Consequently the reaction of soil has a great deal to do with the reaction as well as th temperature.

2. Nitrification.

The nitrification capacity of the soil was determined by WAKSMAN's method[5], and the results obtained are shown in Table VIII. and Fig. VIII.

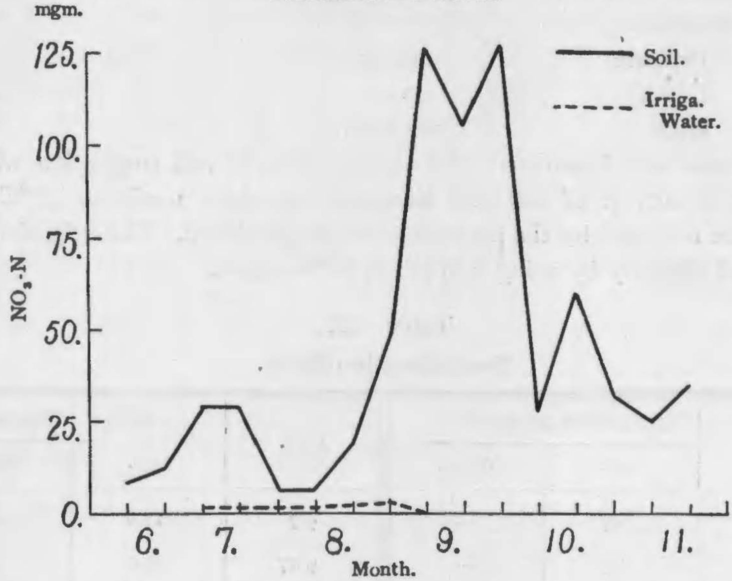
Table VIII.

Nitrification Tests.

Date.	mg. NO_3 in 100 g. Soil.		mg. NO_3 in 100 cc. Water.	
	Control.	Test.	Control.	Test.
6/20	1.82	8.20	—	—
6/29	1.46	13.13	—	—
7/9	1.63	28.36	0.90	0.63
7/19	5.40	27.06	3.00	0.60
7/29	3.20	6.41	0.56	0.44
8/8	2.78	6.34	—	—
8/18	1.06	20.50	—	—
8/28	1.67	50.85	1.67	1.56
9/7	7.12	126.81	0.15	0.19
9/17	1.15	110.31	—	—
9/27	5.25	126.93	—	—
10/7	0.63	28.39	—	—
10/17	3.01	61.55	—	—
10/27	0.43	32.36	—	—
11/7	0.65	25.15	—	—
11/17	0.32	34.19	—	—

1) WAKSMAN, S. A., Soil Science, 15, 57, 1923.
2) HARPER, H. J., Soil Science, 18. 409. 1924.
3) RUSSELL, H. J., and others, Jour. Agr. Science, 3, 111, 1909.
4) SUBRAHMANYAN, ibid, 17, 435, 1927.
5) WAKSMAN, S. A., Soil Science, 16, 57, 1923.

Fig. VIII.
Nitrification Tests.



The nitrification capacity increased gradually toward the middle of July and the maximum was reached at the begining of September. It fell off markedly after October although it was slightly higher than it was at the begining. The reaction as well as the buffer action of the medium must play an important role since such a great activity is manifested. It seems that the maximum activity of nitrification followed after that of the ammonification. It was reported by BATHAM[1] who undertook the investigation noted that the maximum period came at the latter part of July. No comparison however can be made since so many factors such as the amount and kind of fertilizers applied, the crop as well as the cultivation influence the process.

In general, however, the nitrification serves as an index of soil fertility and runs in parallel so far as the previous investigations[2] indicate.

In case of the irrigation water, the activity seems to be very feeble in comparison with that of the soil.

3. Denitrification.

It is extremely interesting to know how intensively the denitrification takes place under the rice-field condition where the anaerobic condition prevails throughout the year.

1) BATHAM, H. N., Soil Science, 20, 348, 1925.
2) WAKSMAN, S. A., Soil Science, 16, 57, 1923.

The determination was carried out by using the following medium in Smith's fermentation tube and the free gas accumulated in the arm was measured as usual:

Peptone	0.1 g.
KNO_3	0.1
H_2O	100.0 cc.

The tube was inoculated with one cc. of 10% soil suspension which is equivalent to 0.01 g. of soil and incubated for three weeks at 28°C. The results were recorded by the percentage of gas produced. The irrigation water was treated similarly by using 0.01 cc. as an inoculum.

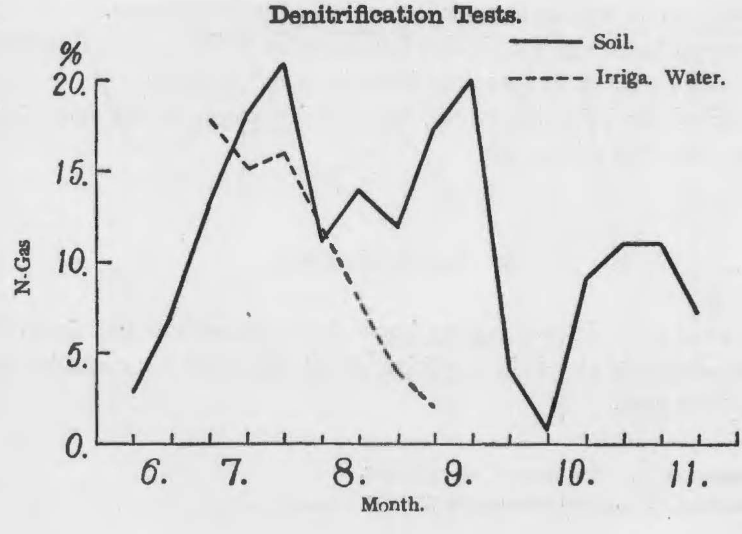
Table IX.

Denitrification Tests.

Date.	% Gas produced.		Date.	% Gas produced.	
	Soil.	Water.		Soil.	Water.
6/20	2.7	—	9/7	17.6	2.3
6/29	6.7	—	9/17	20.0	—
7/9	12.6	18.0	9/27	4.4	—
7/19	17.7	14.7	10/7	0.6	—
7/29	20.9	16.0	10/17	9.0	—
8/8	11.0	—	10/27	11.3	—
8/18	14.1	—	11/7	11.0	—
8/28	12.1	4.3	11/17	7.2	—

Fig. IX.

Denitrification Tests.



As it was naturally expected that the denitrification capacity is quite strong through the months of July and August while the field was covered with the water. It is noteworthy also that the nitrification was very marked in the irrigation water itself. Considering the nature of curve in respect to the ammonification and nitrification, they seem to run in parallel.

4. Nitrogen Fixation.

The fixation capacity was determined by WAKSMAN's method[1] in Ashby's solution, and the results are given in Table X. and Fig. X.

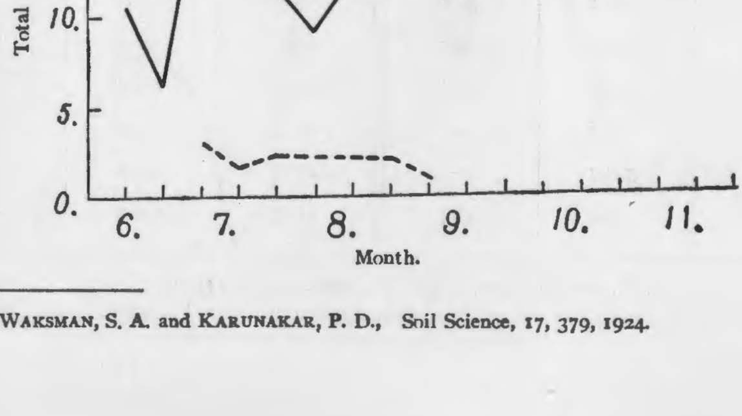
Table X.

Nitrogen Fixation Tests.

Date.	mg. N. fixed.		Date.	mg. N. fixed.	
	Soil.	Water.		Soil.	Water.
6/20	10.3	—	9/7	11.7	1.0
6/29	6.0	—	9/17	11.1	—
7/9	16.9	3.2	9/27	14.3	—
7/19	19.8	1.8	10/7	13.6	—
7/29	11.6	2.3	10/17	14.0	—
8/8	9.2	—	10/27	12.3	—
8/18	11.7	—	11/7	12.6	—
8/28	13.7	2.1	11/17	17.3	—

Fig. X.

Nitrogen Fixation Tests.



1) WAKSMAN, S. A. and KARUNAKAR, P. D., Soil Science, 17, 379, 1924.

As Table X. and Fig. X. indicate that the fixation of nitrogen reached maximum in July and continued throughout the season with a slight variation. As it will be shown in another paper which will report the results of direct examination for Azotobacter by WINOGRDSKY's method, there are numerous Azotobacter present in the soil under question. In case of the irrigation water, the fixation is very much less than that of the soil and amounts to about one fifth or sixth.

5. Cellulose Fermentation.

The cellulose fermentation capacity was determined as follows:

Culture medium; (1)

K_2HPO_4	1.0 gm.
$MgSO_4$	1.0
NaCl	1.0
$CaCO_3$	2.0
$(NH_4)_2SO_4$	2.0
H_2O	1000.0 cc.
Filter paper (fine pieces.)	0.4%

The above medium was inoculated with 1 cc. of 10% soil suspension, equivalent to 0.1 g. of soil and incubated for fourteen days at 28°C. In case of the irrigation water, one cc. was used as an inoculum. At the end of the period, the remainder of filter paper was determined with Schweitzer's reagent after Charpentier[1]. The results are given below:

Table XI.

Cellulose Fermentation.

Date.	mg. cellulose fermented.		Date.	mg. cellulose fermented.	
	Soil.	Water.		Soil.	Water.
6/20	240.8	—	9/7	260.8	52.6
6/29	191.9	—	9/17	279.2	—
7/9	371.4	39.4	9/27	280.0	—
7/19	376.5	36.7	10/7	292.5	—
7/29	376.5	64.1	10/17	274.3	—
8/8	335.3	—	10/27	159.4	—
8/18	303.7	—	11/7	143.1	—
8/28	330.8	47.4	11/17	139.3	—

1) BRADLEY, L. A., and RETTGER, L. F., Jou. Bact., 13, 321, 1927.

2) GRAY, P. H. and CHALMERS, C. H., Ann. Applied. Biol., 11, 330, 1924.

Fig. XI.

Cellulose Fermentation.

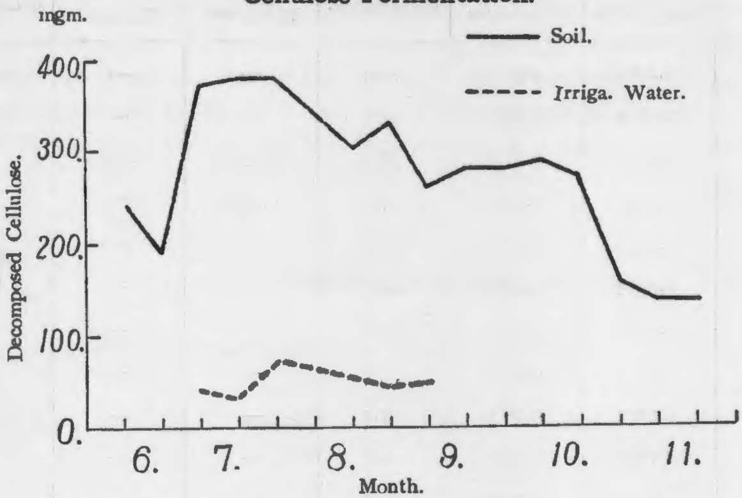


Table XI. and Fig. XI. indicate that the cellulose fermentation was at its maximum at the begining of July and the activity was kept up for a month and gradually decreased in September. The activity in the water was rather feeble.

6. Evolution of Carbon Dioxide.

The determination was carried out by the method recommended by WAKSMAN and STARKEY[1] who determined the respiratory power and decomposition power of the soil. The results are shown below :

Table XII.

Evolution of Carbon Dioxide.

Date.	g. CO_2 produced.		% glucose decomposed.	g. CO_2 produced.		% glucose decomposed.
	Orig. soil.*	Glucose.**		Water.***	Glucose.	
6/20	0.0927	0.5126	51.0	—	—	—
6/29	0.1235	0.4341	43.8	—	—	—
7/9	0.1932	0.6543	58.0	0.1441	0.0874	11.9
7/19	0.1440	0.7231	58.2	0.0386	0.0734	10.0
7/29	0.1363	0.8553	68.8	0.0397	0.0482	6.6

1) WAKSMAN, S. A. and STARKEY, R. L., Soil Science, 17, 141, 1924.

Date.	g. CO_2 produced.		% glucose decomposed.	g. CO_2 produced.		% glucose decomposed.
	Orig. soil.*	Glucose.**		Water.***	Glucose.	
8/8	0.1081	0.5912	52.4	—	—	—
8/18	0.0809	0.5662	52.5	—	—	—
8/28	0.1432	0.7169	57.0	0.0380	0.0460	6.3
9/7	0.1211	0.7100	57.5	0.0341	0.0228	3.2
9/17	0.1210	0.5262	45.6	—	—	—
9/27	0.0954	0.4853	45.0	—	—	—
10/7	0.1412	0.4391	41.2	—	—	—
10/17	0.2061	0.4030	35.3	—	—	—
10/27	0.1355	0.2686	25.4	—	—	—
11/7	0.1387	0.2200	20.8	—	—	—
11/17	0.1368	0.1749	17.3	—	—	—

* 100 grams of soil was taken.

** 0.5 gram of glucose was added to 100 grams of soil.

*** 100 cc, of irrigation water was used.

Fig. XII.

Evolution of Carbon Dioxide.

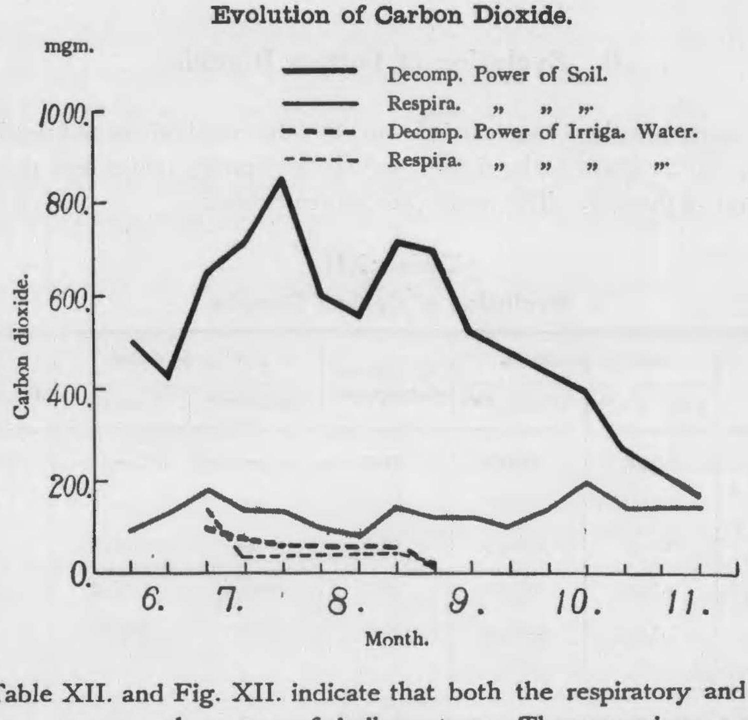


Table XII. and Fig. XII. indicate that both the respiratory and decomposition power, gave the curves of similar nature. The power increased steadily from the begining of July while the latter decreased slightly for a while. At the middle of November, both powers came close to each other. This may indicate that the growth of plants as well as the change of microflora in the soil.

The CO_2 production from the soil has been considered as an index of microbiological activity: PETERSEN[1], in 1870, WOLLNY[2] in 1897 had tested the phenomenon, and they were followed by numerous other workers. Recently WAKSMAN and STARKEY[3] showed the parallel relation among the respiratory power and number of bacteria, nitrification and crop productivity. The two powers however are influenced by the kind of fertilizers used. For instance when the ammonium sulfate is applied, the respiratory power usually decreases owing to the increase of activity brought about by the fertilizer. Thus enables the increase growth of fungi flora so that the decomposition power increases markedly.

Summary and Conclusions.

From the results obtained in this investigation, the following summary and conclusions may be given:

Part I. *Ecological Factors:*

1. The temperature of irrigation water and at the soil surface reached as high as 34°C. which is four to five degrees higher than that of the air up to Aug. 20th, after that the atmospheric temperature gradually became higher than the others.

2. It was rather dry season during June and July, but in September there was 170 mm. rainfall and the rest of the season was normal, averaging 29 mm. rainfall.

3. The moisture content of the soil was 41% while the field was irrigated, and at other times, 26—39%.

4. The concentration of hydrogen ions in the soil varied between pH 5.90—7.25. Soon after the application of fertilizers, the reaction became alkaline pH 7.25 and toward the end of season, it became slightly acid pH 5.90. On the other hand, the irrigation water remained alkaline all the way through, pH 7.32—7.94.

Part II. *Microbiological Investigation:*

1. One gram of soil contains 2151×10^4 of bacteria on an average, having a range of $66—3511 \times 10^4$, and the large number of them were found in June

1) PETERSEN, P., Die Landw. Versuch, 13, 155, 1870.

2) WOLLNY, E., Die Zersetzung d. Organischen Stoff u. d. Humusbildung. Berlin, 1897.

3) WAKSMAN, S. A. and STARKEY, R. L., loc. cit. 1924.

and October. The same amount of soil contained $0.3-5 \times 10^4$ of fungi.

2. One cc. of irrigation water contained about 8×10^4 of bacteria and $0.02-2 \times 10^4$ of fungi.

3. The ammonification capacity of the soil was strongest at the end of August and the begining of September while it was very feeble in case of the irrigation water.

4. The nitrification capacity gradually increased toward the middle of July and the maximum was reached at the begining of September. It was feeble in case of the irrigation water.

5. The denitrification capacity is quite strong through the months of July and August while the field was covered with water. It was very marked in the irrigation water itself. The nature of curves seems to run in parallel with those of ammonification and nitrification.

6. The fixation of nitrogen of the soil reached its maximum in July and continued throughout the season with slight variation. The fixation was very much less in the irrigation water than that of the soil.

7. The cellulose fermentation was at its maximum at the begining of July and the activity was kept up for a month and gradually decreased in September. The activity in the irrigation water was rather feeble.

8. The respiratory and decomposition powers of the soil was determined and found that the former increased steadily from the begining of July while the latter decreased slightly for a while. At the middle of November, both powers came close to each other which may be due to the change of microflora in the soil.

Further each one of these microbial activities will be considered in different plots, one by one, so that the comparative results may be obtained in respect to the ecological conditions.