

## THE LENGTH OF TIME TO INCUBATE PETRI PLATES.

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Investigations of the steps entering into the plate method for the enumeration of the number of bacteria present in soil are few. So little agreement was observed in the procedures followed in different soil bacteriology laboratories that investigations were undertaken in this station to develop a reasonably accurate technic for the bacteriological examination of soils.<sup>1</sup> The present paper gives data in support of the ten-day period of incubation at 20° C. for soil plates. The work was done jointly with that on methods of sampling soil for bacteriological analysis<sup>2</sup> and the number of colonies satisfactory for a petri plate.<sup>3</sup> Among the soil factors considered in connection with the length of time to incubate plates were the kind of soil, the nature of its flora, temperature when sampled, the uniformity of sampling, the moisture content, and the condition of aeration.

*It was early decided that probably the chief reason why confidence is lacking in the significance of plate counts is because the organisms have not usually been given the proper chance to develop into colonies.*

Table I has been prepared to show how differently organisms develop into colonies under different periods of incubation. The technic followed was that previously described,<sup>1</sup> and the figures are based on the average of three plates in each case.

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<sup>1</sup> Noyes, H. A., and Voigt, Edwin, in *Proceedings of Indiana Academy of Science*, 1916, pp. 272-301.

<sup>2</sup> Noyes, H. A., in *Journ. Amer. Soc. of Agron.*, No. 5, 1915, pp. 239-249.

<sup>3</sup> Noyes, H. A., and Grounds, G. L., in *Proceedings of Indiana Academy of Science*, 1918.

TABLE I.  
COVER CROPPED SOILS

Percent Counts at 3, 5, and 7-Days Incubation are of 10-Days Counts.\*

CROPPED TO	BACTERIAL DILUTION							
	1-40,000				1-100,000			
	3	5	7	10 Days	3	5	7	10 Days
<i>Nothing</i>								
November 14, 1914.....	30.1%	55.9%	**%	100.0%	**%	52.2%	**%	100.0%
February 6, 1915.....	15.5	74.4	88.1	100.0	43.1	62.6	62.6	100.0
March 2, 1915.....	56.4	84.1	93.4	100.0	42.9	88.4	86.4	100.0
March 27, 1915.....	25.3	54.4	68.9	100.0	12.5	46.3	58.8	100.0
April 15, 1915.....	13.4	66.2	85.2	100.0	29.8	70.2	93.0	100.0
Average....	27.8	67.0	83.9	100.0	32.1	63.9	75.2	100.0
<i>Millet</i>								
November 14, 1914.....	33.2	58.4	**	100.0	**	55.9	**	100.0
February 6, 1915.....	21.9	84.6	100.0	100.0	24.1	60.3	56.4	100.0
March 2, 1915.....	48.6	94.6	93.1	100.0	33.8	77.5	87.5	100.0
March 27, 1915.....	32.8	79.2	90.6	100.0	14.2	54.3	80.0	100.0
April 15, 1915.....	26.7	82.6	92.2	100.0	7.4	67.9	82.7	100.0
Average....	32.6	80.0	94.0	100.0	19.9	63.2	76.7	100.0
<i>Soy Beans</i>								
November 14, 1914.....	18.6	42.9	**	100.0	**	54.8	**	100.0
February 6, 1915.....	33.0	65.4	80.8	100.0	18.5	54.2	66.7	100.0
March 2, 1915.....	49.4	88.2	99.4	100.0	36.5	74.6	88.8	100.0
March 27, 1915.....	30.0	70.9	88.2	100.0	22.6	61.3	58.1	100.0
April 15, 1915.....	12.5	85.7	98.2	100.0	11.1	75.0	91.5	100.0
Average....	26.9	70.6	91.7	100.0	22.2	64.0	76.3	100.0
<i>Nothing</i>								
November 14, 1914.....	20.3	54.5	**	100.0	**	60.0	**	100.0
February 6, 1915.....	53.2	97.6	100.0	100.0	35.3	85.3	88.2	100.0
March 2, 1915.....	44.1	84.3	83.1	100.0	14.3	71.4	81.4	100.0
March 27, 1915.....	36.2	77.3	91.4	100.0	20.1	50.0	67.6	100.0
April 15, 1915.....	18.8	73.3	78.4	100.0	15.8	73.7	100.0	100.0
Average....	34.5	77.4	88.2	100.0	21.4	68.1	84.3	100.0
<i>Hairy Vetch</i>								
November 14, 1914.....	19.6	40.1	**	100.0	**	42.3	**	100.0
February 6, 1915.....	30.7	63.8	84.4	100.0	20.3	54.7	85.9	100.0
March 2, 1915.....	43.8	83.2	97.2	100.0	20.2	73.7	88.5	100.0
March 27, 1915.....	28.1	71.4	100.0	100.0	28.5	69.4	89.1	100.0
April 15, 1915.....	15.3	84.9	94.1	100.0	29.5	68.2	88.6	100.0
Average....	27.5	69.7	93.9	100.0	24.6	61.7	88.2	100.0
<i>Winter Rye (Sown Early)</i>								
November 14, 1914.....	26.5	55.2	**	100.0	**	35.5	**	100.0
February 6, 1915.....	46.1	48.9	75.1	100.0	16.5	49.5	53.6	100.0
March 2, 1915.....	46.5	63.2	79.4	100.0	43.4	84.9	84.9	100.0
March 27, 1915.....	30.3	76.4	86.9	100.0	22.4	44.9	65.3	100.0
April 15, 1915.....	24.5	84.8	88.0	100.0	10.8	63.8	86.8	100.0
Average....	34.8	65.7	82.4	100.0	23.3	66.7	72.7	100.0
<i>Nothing</i>								
November 14, 1914.....	25.1	57.3	**	100.0	**	34.5	**	100.0
February 6, 1915.....	22.9	68.3	74.7	100.0	38.2	77.9	94.1	100.0
March 2, 1915.....	61.8	83.7	101.6	100.0	32.7	69.2	90.4	100.0
March 27, 1915.....	23.5	56.7	77.5	100.0	23.7	64.3	61.9	100.0
April 15, 1915.....	8.3	42.3	52.7	100.0	13.3	73.3	97.7	100.0
Average....	28.3	61.7	76.6	100.0	27.0	63.9	86.0	100.0

TABLE I—Continued.

## COVER CROPPED SOILS

*Percent Counts at 3, 5, and 7-Days Incubation are of 10-Days Counts.\**

CROPPED TO	BACTERIAL DILUTION							
	1-40,000				1-400,000			
	3	5	7	10 Days	3	5	7	10 Days
<i>Winter Rye (Sown Late)</i>								
November 14, 1914	20.9	40.9	**	100.0	**	38.0	**	100.0
February 6, 1915	26.2	85.0	86.4	100.0	20.3	68.5	85.2	100.0
March 2, 1915	52.9	85.6	98.9	100.0	31.1	70.5	83.6	100.0
March 27, 1915	27.6	68.0	82.4	100.0	16.6	43.8	58.3	100.0
April 15, 1915	14.0	66.2	85.2	100.0	20.7	54.8	86.6	100.0
Average	28.3	63.1	88.2	100.0	22.2	55.1	78.4	100.0
<i>Crimson Clover</i>								
November 14, 1914	20.5	41.3	**	100.0	**	44.9	**	100.0
February 6, 1915	36.6	91.2	99.6	100.0	26.5	44.9	65.3	100.0
March 2, 1915	55.8	86.9	93.6	100.0	36.6	80.5	85.4	100.0
March 27, 1915	26.1	59.4	90.6	100.0	33.3	66.6	66.6	100.0
April 15, 1915	10.3	68.4	85.8	100.0	10.0	78.0	92.0	100.0
Average	29.9	69.4	92.4	100.0	26.6	63.0	77.3	100.0
<i>Nothing</i>								
November 14, 1914	24.7	47.3	**	100.0	**	42.0	**	100.0
February 6, 1915	18.6	86.7	93.5	100.0	9.2	62.9	79.6	100.0
March 2, 1915	50.0	76.5	83.0	100.0	34.6	76.9	73.5	100.0
March 27, 1915	31.7	62.8	95.4	100.0	36.9	44.4	66.7	100.0
April 15, 1915	16.0	76.8	92.3	100.0	17.2	55.2	89.6	100.0
Average	28.2	70.0	91.1	100.0	21.5	56.3	77.4	100.0
<i>Buckwheat</i>								
November 14, 1914	12.0	50.0	**	100.0	**	64.5	**	100.0
February 6, 1915	19.2	94.7	105.2	100.0	25.7	65.1	69.7	100.0
March 2, 1915	40.5	80.7	87.1	100.0	40.5	80.7	91.2	100.0
March 27, 1915	26.7	53.5	77.1	100.0	20.0	55.0	55.0	100.0
April 15, 1915	21.2	77.2	84.1	100.0	11.9	86.6	83.3	100.0
Average	23.9	71.2	88.4	100.0	24.5	66.6	74.8	100.0
<i>Natural Growth of Weeds</i>								
November 14, 1914	22.5	53.1	**	100.0	**	64.7	**	100.0
February 6, 1915	31.2	85.0	100.0	100.0	31.9	75.0	84.7	100.0
March 2, 1915	55.1	73.7	85.8	100.0	35.4	70.8	72.6	100.0
March 27, 1915	27.1	71.3	88.3	100.0	20.5	55.9	73.5	100.0
April 15, 1915	22.5	81.7	96.0	100.0	17.1	85.3	97.6	100.0
<i>Averages</i>								
November 14, 1914	22.8	49.7	**	100.0	**	49.1	**	100.0
February 6, 1915	29.6	78.8	90.7	100.0	25.8	63.4	74.3	100.0
March 2, 1915	48.7	80.8	91.7	100.0	34.9	80.3	84.5	100.0
March 27, 1915	28.8	66.8	86.4	100.0	22.5	54.7	66.5	100.0
April 15, 1915	16.9	74.2	86.0	100.0	16.2	69.3	90.8	100.0
<i>Averages of All</i>	31.7	74.0	92.5	100.0	26.2	70.3	82.1	100.0

\*\*Counts not made.

\*Temperature of Incubation 20° C.

The variations between the per cent of the colonies that developed is from 7.4 to 43.4 per cent for the three days' incubation, 34.5 to 88.4 per cent for the five days' incubation, and from 53.6 to 100.0 per cent for the seven days' incubation. The figures are taken from the 1-400,000 bacterial dilutions, where the number of colonies was small enough to allow for all organisms to develop into colonies. The plates for the 1-40,000 bacterial dilutions in many cases had too many organisms for satisfactory counts, and this is shown in the general averages for this dilution as compared to those for the 1-400,000 bacterial dilution. The cropping system, the aeration of the soil and soil temperature very evidently influence the rate at which the organisms of soil develop into discernable colonies on petri plates.

One contention for the use of the bacteriologist's soil sampler<sup>2</sup> was that it sampled the soil accurately to the depth desired and kept the sample under field conditions of aeration until analyzed. Table II gives data showing how the methods of sampling can be compared by the relative distribution of the rapid and slow growing organisms present in the different samples.

TABLE II.

Percent 3, 5, and 7-Day Counts of 10-Day Counts \*\*.  
(Gravelly soil sodded and containing about 7% moisture.)

TIME OF INCUBATION		3	5	7	10 Days*
Bacteriologist's Soil Sampler	1	15.3%	20.8%	53.8%	100.0%
	2	11.8	39.4	49.0	100.0
	3	11.1	26.1	46.3	100.0
	Average.....	12.7	28.8	49.7	.....
P. E. Brown's Method	1	11.5	46.0	72.8	100.0
	2	29.2	43.8	65.7	100.0
	3	14.1	26.4	45.0	100.0
	Average.....	18.3	38.4	61.2	.....
Slice Method	1	20.5	48.2	64.0	100.0
	2	4.8	42.8	63.2	100.0
	Average.....	12.7	45.5	63.6	.....
Average of all.....	14.8	37.7	57.4	.....	

\*Counts after 10 days incubation at 20°C taken as 100% those at other times are stated as parts of this. Bacterial Dilution 1-400,000.

\*\*Counts were about 3.0 million per gram of dry soil.

This test showed:

1. That the organisms present in this packed sodded land were principally slow growers.
2. That the uniformity of the development of colonies varied with the method by which the samples were drawn.

We have found by numerous tests that the number of organisms found in sodded soil at or below a depth of four inches is much less than nearer the surface; and, further, it has been observed that those organisms occurring at the lower depths do not usually multiply as rapidly on aerobic plates as those occurring nearer the surface. The samples procured with the bacteriologist's soil sampler evidently had near their proper proportions of slowly multiplying organisms.

In testing out the quantities of soil necessary for bacteriological examinations some tests were made with air-dry samples to show that even when samples were unified by air-drying a large quantity was necessary for accurate results. Table III gives the development of colonies after different periods of incubation on air-dry soil sieved to pass 1 millimeter, while Table IV gives results secured on the same sample of air-dry soil when further unified by using only that portion passing a sixty-mesh sieve.

TABLE III.  
*Percent 5 and 7-Day Counts are of 10-Day Counts.*  
*(Air Dry Loam Soil, sieved C pass 1 mm.)*

TIME OF INCUBATION		5	7	10 Days*
Sample No.	Size of Sample			
1.....	50 grams	72.7%	92.1%	100.0%
2.....	50 grams	79.4	91.2	100.0
3.....	50 grams	73.0	88.6	100.0
4.....	10 grams	87.9	95.0	100.0
5.....	10 grams	95.4	96.8	100.0
6.....	10 grams	58.8	85.8	100.0
7.....	5 grams	71.2	91.8	100.0
8.....	5 grams	67.8	85.2	100.0
9.....	5 grams	75.8	97.1	100.0
10.....	1 gram	86.4	95.2	100.0
11.....	1 gram	72.1	80.5	100.0
12.....	1 gram	74.9	80.3	100.0
13.....	0.5 gram	64.7	85.0	100.0
14.....	0.5 gram	79.3	85.2	100.0
15.....	0.5 gram	62.5	79.4	100.0
	Average.....	74.8	88.6	100.0

\*Counts after 10 days incubation at 20° C taken as 100.0%.  
 Other counts states as parts of these.  
 Bacterial Dilution 1-400,000.

TABLE IV.

Percent 5 and 7-Day Counts are of 10-Day Counts.  
(Air Dry Loam sieved to pass 60 mesh.)

TIME OF INCUBATION		5	7	10 Days*
Sample No.	Size of Sample			
1.....	50 grams	79.9%	96.8%	100.0%
2.....	50 grams	67.0	100.0	100.0
3.....	50 grams	89.8	100.0	100.0
4.....	10 grams	63.7	72.1	100.0
5.....	10 grams	72.6	91.0	100.0
6.....	10 grams	74.7	100.0	100.0
7.....	5 grams	85.2	93.2	100.0
8.....	5 grams	90.8	95.7	100.0
9.....	5 grams	63.4	73.2	100.0
13.....	1.0 gram	93.7	100.0	100.0
14.....	1.0 gram	63.6	75.3	100.0
15.....	1.0 gram	62.8	85.8	100.0
16.....	0.5 gram	64.3	73.7	100.0
17.....	0.5 gram	66.5	100.0	100.0
18.....	0.5 gram	74.9	77.5	100.0
	Average.....	74.2	88.9	100.0

\*Counts after 10 days incubation at 20°C taken as 100%.

Other counts stated as parts of these Bacterial dilution 1-100,000.

The results given in the previous tables show:

1. That the greater proportion of the organisms present in this air-dry soil develop into colonies after five days' incubation.
2. The larger the aliquot of soil used the more uniformity between the development of colonies on the plates.
3. In five cases out of the fifteen all the colonies were counted after seven days' incubation when the soil was sieved to pass a sixty-mesh sieve.

It has been observed, in soil bacteriology investigations in an apple orchard where different systems of soil management are practiced, that the organisms multiply into colonies at different rates, dependent on the system of management practiced. The results of this work are given in Table V.

TABLE V.

*Average Percent 5 and 7-Day Counts are of 10-Day Counts.  
(Silt Loam Subjected to Different Systems of Soil Management.)*

TIME OF INCUBATION		5	7	10 Days*
Clean Cultivation	1	41.9%	100.0%	100.0%
	2	27.2	97.3	100.0
	3	23.5	100.0	100.0
Sod	1	42.3	82.3	100.0
	2	41.5	78.8	100.0
	3	33.3	79.0	100.0
Straw Mulch	1	65.1	85.3	100.0
	2	55.2	87.4	100.0
	3	65.2	81.7	100.0
Light Grass Mulch	1	67.3	88.2	100.0
	2	58.3	96.9	100.0
	3	37.0	82.2	100.0
Average All.....		46.5	88.3	100.0
Average Clean Cultivation.....		30.9	99.1	100.0
Average Sod.....		39.0	80.0	100.0
Average Straw Mulch.....		61.8	84.8	100.0
Average Light Grass Mulch.....		54.2	89.1	100.0

Table V shows:

1. The rate of development of colonies varies with the system of soil management.
2. Those conditions which unify differences in soil aeration are present where the rates of development of colonies check closest.
3. Short periods of incubation would not show the relative numbers of bacteria actually present in the soils.

Many sets of plates have been counted after twelve and fifteen days' incubation, but very rarely have counts increased at all after ten days' incubation. With suitable media the counts obtained after seven days' incubation have uniformly shown the comparisons between samples, and this does not mean that the increases from seven to ten days are numerically or proportionately the same.

SUMMARY.

Counts made after ten days' incubation at 20° C. of petri plates, made from bacterial dilutions of soil, give reliable results as to the bacterial content of the soil, providing the number of colonies present per plate is small enough for all organisms to develop into colonies.

The rapidity with which bacteria develop into colonies has been shown to vary with the soil, and to be influenced by soil temperature, moisture and aeration.

Much of the lack of confidence in results obtained by the plate method is due to having too many colonies present per plate<sup>3</sup> and not allowing sufficient time of incubation of the petri plates.