

## BACTERIA IN FROZEN SOIL.

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Two soil bacteriologists have published data as showing that the number of bacteria in soil increases when the soil is frozen. These reported increases in numbers are so contradictory to general belief concerning bacterial activities at low (about freezing) temperatures that not only the experimental data but abstracts of the technic followed are given below.

Figure 1 gives the data presented in Cornell University Agricultural Experiment Station Bulletin No. 338. The following is an abstract of the technic followed:

"Samples of soil were usually taken with an auger or by the combined use of an auger and pick when the ground was frozen. During the winter of 1909-1910 a pick alone was used. When an auger was employed the proceeds from two or three borings were combined, except in winter, when only one hole was made; but when the pick alone was used it was impossible to take any such pains in order to obtain a representative sample. \* \* \* The depth of sampling was six to eight inches, although in winter 1909-1910 it varied more than during the remainder of the period. \* \* \* The soil was carefully mixed, in summer by sieving through a sieve as fine as the moisture content would allow, in winter by stirring after thawing. Of this soil 0.5 gram was added to sufficient sterile water to make a volume of 100 cc. \* \* \* The samples taken from any one of these four spots must have all been from within a circle of six-inch radius. The media used varied; the one most extensively used was soil extract gelatin containing 0.1 per cent dextrose. Plates were incubated seven days at 19° to 20° C. for gelatin and usually two weeks for agar."

The following statement is taken from the author's summary of the work<sup>1</sup>: "Quantitative determinations \* \* \* have shown \* \* \* an increase in numbers of bacteria in frozen soil."

Figure 2 gives the data presented in Research Bulletin No. 4 of the Iowa Experiment Station. The following is an abstract of the technic followed:

"The samples were drawn from the plot already described within an area of about five feet square. \* \* \* They were taken to a

<sup>1</sup> Conn. H. J., in *Centrab't fur Bakt II* Abteil. 28 (1910), p. 422.

depth of 20 cm. by means of a 2.5-inch auger, except during the time that the soil was frozen, when it became necessary to substitute a mattock or grub hoe for the auger. The samples were collected on a sterile mixing cloth and then placed in sterile glass jars and taken to the laboratory and inoculations performed as quickly as possible. \* \* \* In this work it was deemed inadvisable to permit such a multiplication of organisms to occur in the sample as would undoubtedly take place if they were allowed to stand long enough to thaw out completely. Consequently the frozen samples employed here were thoroughly comminuted by means of a sterile spatula, carefully mixed, and then subsampled for inoculations. The maximum time required to prepare the sample in this way was ten minutes. \* \* \* 100 gram quantities of the soil prepared \* \* \* were shaken for five minutes with 200 cc. portions of sterile distilled water. Lipman and Brown 'synthetic agar' was used and counts made after three days' incubation at 22° C. Results are averages of two dilutions which agreed closely in every case."

The author summarizes the results given in Figure 2 as follows:

1. "By means of the 'modified synthetic' agar plate method, bacteria are shown to be present in large numbers in a typical Wisconsin drift soil when it is completely frozen and the temperature is below zero degrees Centigrade; furthermore, increases and decreases in numbers of organisms occur during this period and larger numbers are found after the soil has been frozen for a considerable period than before it begins to freeze."

2. "During the fall season, the number of bacteria present in the soil diminishes gradually with the lowering of the temperature."

The methods of sampling and the technic employed in getting the results reported in the above mentioned publications were so different from those adopted in this laboratory, after much testing, that the results of data on bacterial counts obtained on different dates from samples of a silt loam variously cover cropped are given in Figure 3. The technic of sampling, diluting and plating is that previously described.<sup>2</sup>

It is to be noted that the numbers of bacteria found in the soil when the temperature was 32° or lower were greater than those found at other times during the winter. The soil thermometers were at a depth of nine inches and the samples were drawn to this depth. It had been found impracticable to take samples when the ground was solidly frozen, and samples were taken (on the dates) started just as the soil had thawed enough so that the samplers<sup>3</sup> could be used. The question thus

<sup>2</sup> Noyes, H. A., Voigt, Edwin, in Proceedings Indiana Academy of Science, 1916, pp. 272-301.

<sup>3</sup> Noyes, H. A., in Journ. Amer. Soc. Agron. Vol. 7, No. 5 (1915).

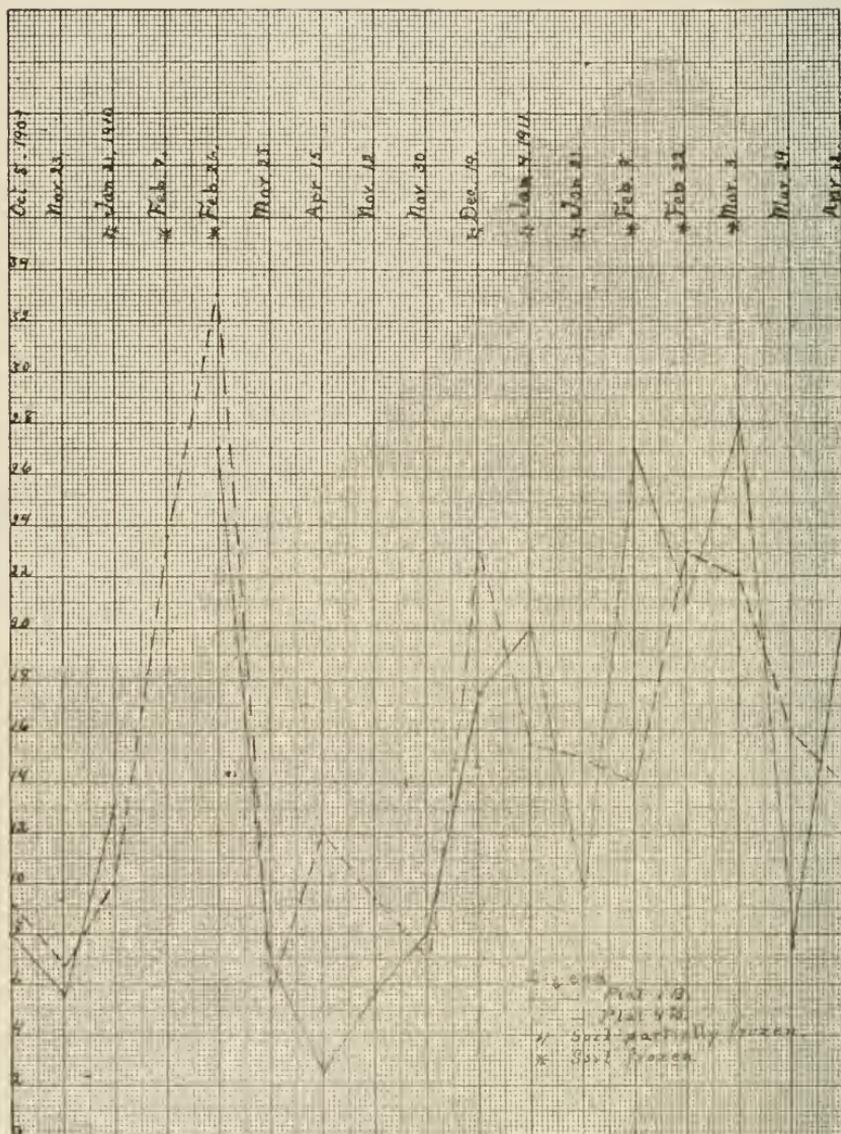


Figure 1. Bacteria Counts, 20 million per c.c. of water, New York.  
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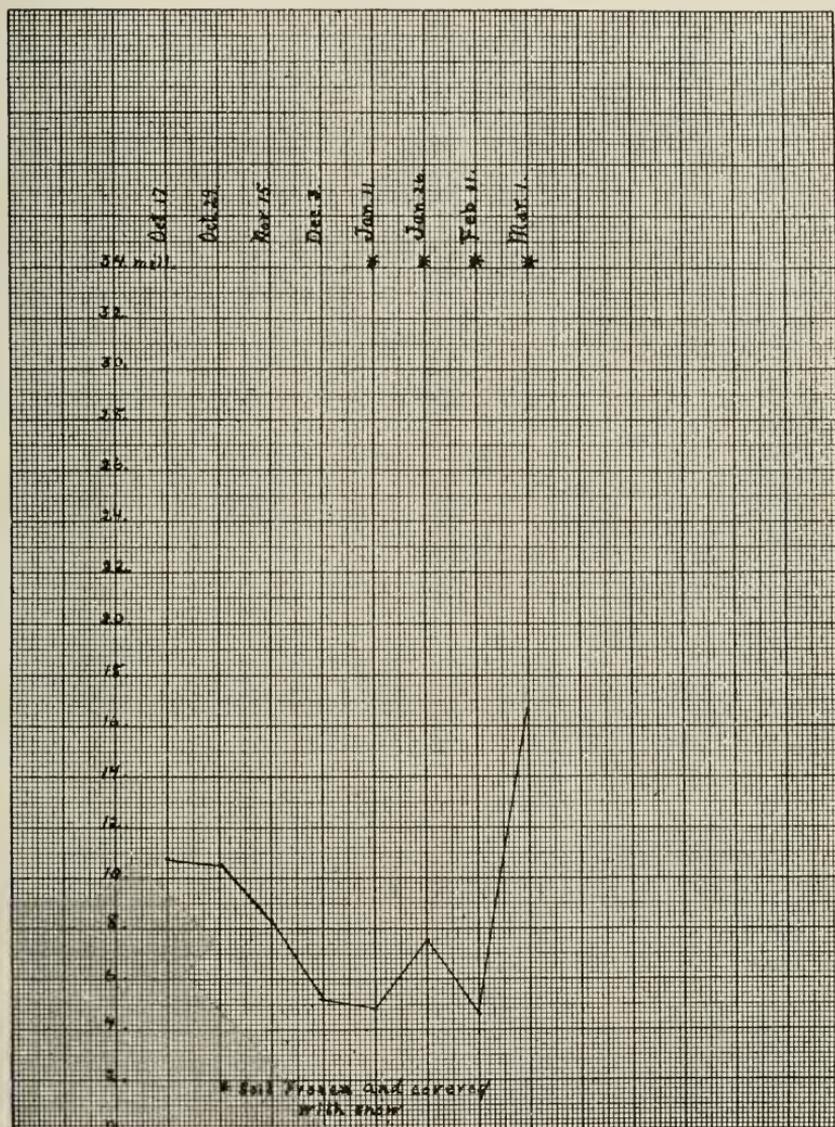


Figure 2 Bacterial Counts per gram of dry soil, Iowa.

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naturally arose as to whether the counts obtained in this and the previous work were not due to increased bacterial activities as the ground thawed.

To give more definite information, special experiments have been carried out. A special bacteriologist's soil sampler<sup>3</sup> reinforced with steel was secured and driven down into solidly frozen soil. The sampler containing the frozen soil was brought into the warm laboratory and in a half hour it was possible to push the core of frozen soil out of the sampler. This core was placed on a laboratory table. A wire was pushed into the core from time to time and it was found that thawing took place very slowly. It was forty-six hours from the time that the sample was laid out on the table before it had thawed enough for the wire to be thrust through it.

To see if the bacterial numbers in soil were not increased on the thawing out of the soil due to different layers of the soil being brought successively under more favorable conditions for bacterial development, the following test was made:

A sample of frozen loam soil was obtained, brought to the laboratory, pushed out of the sampler, then taken to a room having a temperature below 0° C., where it was halved lengthwise by chopping with an axe. One-half was chopped and mixed and fifty grams weighed out and analyzed *immediately* for its bacterial content. The other half was brought to the laboratory and allowed to stand twenty-four hours. It thawed out in this time. The sample was mixed and its bacterial content determined. The results of this test were that the sample allowed to thaw out before it was analyzed gave over three times the bacterial count that the one analyzed immediately did.

The following experiment is the latest one we have conducted on this subject, and it is left to the reader to judge from this in connection with the other work reported as to whether bacteria multiply in frozen soil. About twenty kilos of soil (silt loam) were procured by taking soil from between the depths of four and seven inches of a plot where millet had been plowed under each of the two preceding springs. This soil was mixed and sieved through a screen having eight meshes to the inch. The portion passing the screen was mixed thoroughly and then quartered. One quarter (about five kilos) was brought to the laboratory. Sterile 12-ounce bottles plugged with cotton had been previously prepared and 150 grams of the mixed and prepared sample were weighed out into each of twenty-six bottles. The soil in the bottles was then compacted by dropping them on the bench thirty times. The bottles were then divided into three groups and these groups were incubated in the following places:

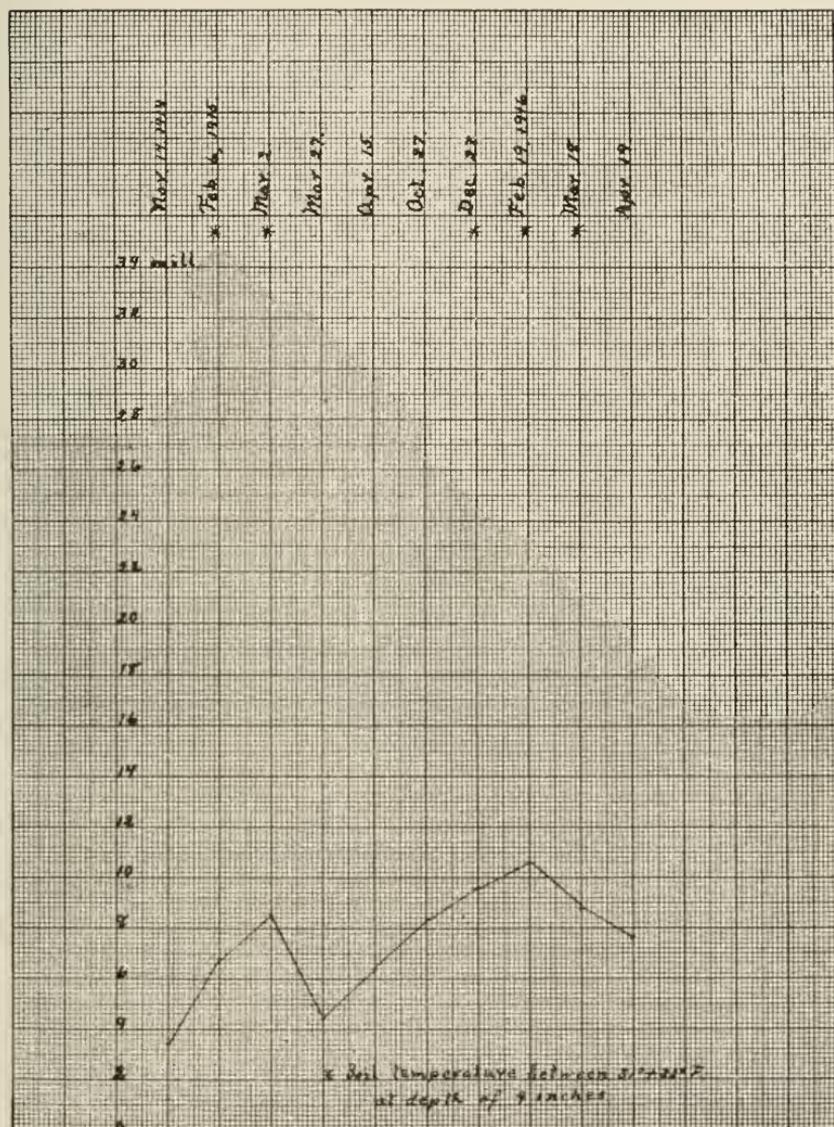


Figure 3 Bacterial Counts in millions per gram dry soil  
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 Indiana.

- A. An ice box, temperature around 45° F.
- B. In a cold storage room in a creamery, temperature 36° F. to 42° F.
- C. Cold room in creamery, temperature between 27° F. and 30° F.

The counts obtained from these tests are given in Table I.

TABLE I.

*Changes in Bacterial Content of Soil Stored in Different Refrigerating Rooms.*

Lengths of Time of Incubation	Temp. 45° F	Temp. 39° F	Temp. 29° F
0 days	12.4*	12.4	12.4
21	11.7	9.5	5.5
78	0.7	4.8	4.5

\*Figures are millions per gram of soil as used.

## SUMMARY.

It is known to be difficult to get accurate figures of the numbers of bacteria present in frozen soil. It is not known that the layer of soil just below the constantly increasing layer of frozen soil is not very favorable for the multiplication of *certain* classes of bacteria.

The data reported in this paper, obtained in this laboratory and from the work of others does not prove that the number of bacteria present in soil is increased when the soil is frozen.