EXPERIMENT STATION

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N. S. MAYO, M. S., D. V. S. . . . .Veterinarian.
A. T. KINSLEY, M. S. . . . . Assistant*

BACTERIA OF THE SOIL.

THE agriculturists of the Eastern states expend large sums annually for fertilizers to maintain the fertility of the soil, and their experiment stations are constantly testing and making chemical analyses of the different artificial fertilizing compounds. The soils of the Western states do not at present require the application of artificial fertilizers to insure a crop, and their experiment stations do not investigate them, neither do they, in most instances, seem concerned in the maintenance of the fertility of the soil. The soil of the Eastern states was formerly as productive as that of the Middle and West at the present time, but by constant cropping the fertility has been partially exhausted. The fertility of the soil of the Middle states and the West is being rapidly diminished and if means are not taken to prevent it, the time is not far distant when it will be as necessary to apply artificial fertilizers to the soil as it is now in the East.

Kansas is an agricultural state. The annual crop production is on the increase. The soil is fertile, although some find it necessary to apply artificial fertilizer. The natural fertility of the soil depends to a large extent upon bacterial activity, and if the soil be cared for properly no artificial fertilization will ever be needed. It is

*The experiments described in this bulletin were carried on almost entirely according to the plans and under the direction of Mr. Kinsley.

more economical to maintain the natural fertility of the soil than to apply artificial fertilizers. By a series of experiments no doubt methods could be determined whereby the bacteria could be increased in the soil and their products utilized in the maintenance of fertility.

Before entering upon the subject proper, it has been deemed advisable first to give a brief description of bacteria. In ordinary parlance, bacteria, or germs, are usually spoken of as antagonistic to the welfare of mankind. This is a popular error. If some species or variety of a certain group of organisms is injurious, it does not follow that the whole group is injurious. Solonacae, an order of the vegetable kingdom, includes the deadly nightshade, jimson and other poisonous plants, as well as the potato and tomato, which are used extensively as food-products. But man is apparently more impressed by his antagonist, and since various disease-producing bacteria have been frequently spoken of in various periodicals, the hasty conclusion has been that all bacteria are injurious. However, our fathers and our forefathers should not be unduly criticized for their ignorance of bacteria, for twenty-five years ago bacteria scarcely received a mention in any of the institutions of learning. Only the advanced medical colleges provided for instruction on disease germs twenty years ago. A decade ago very few agricultural colleges provided for bacteriological instruction, because it was thought that physicians only required a knowledge of bacteria; but now practically all institutions of learning have well-equipped laboratories for the thorough investigation of these friends and foes of man; for, from the recent investigations, it is known that upon a knowledge of their functions depends the future progress of dairying, agriculture, preservation of foods, and many other industries, as well as medicine.

WHAT BACTERIA ARE.— Bacteria are plants. Although they were formerly classed as animals, it is now universally conceded that they are plants. They are single-celled and of simple structure, being composed almost entirely of protoplasm; they differ from higher plants in that they contain no chlorophyll (green coloring matter). They resemble more closely the yeasts and molds. There are many different varieties of bacteria; about 1200 different kinds have been isolated and studied, and new varieties are being found every day.

SIZE.— Bacteria are very small, being invisible to the naked eye. It would take about 10,000 average-sized bacteria placed end to end to make an inch in length, or it would take about one and one-half millions in a cube to make a mass large enough to be seen with the unaided eye.

FOOD, DIGESTION, EXCRETIONS.—The food requirement varies according to the species. As a rule, bacteria, like other non-chlorophyllic plants, require preformed organic food; although some soil bacteria apparently thrive best, or only on mineral foods. Generally speaking, bacterial food resembles closely the food of man; thus, milk, an excellent human food, is also an excellent bacterial food. Bacteria digest their food by ferments secreted by their bodies; the process in general is the same as that of man. The food thus digested is absorbed by the bacteria, a part of it being used in the construction of new protoplasm, and a part to produce energy in the form of heat, motion, and possibly light.

Bacterial secretions or waste products vary with the food, environment, and the organism acting. Vinegar is a by-product or a bacterial excerment. The strong odors produced in putrefaction are products of bacteria action. Sausage and ice-cream poisoning are results of bacteria decomposition. In general, the excrements of bacteria are injurious to their own bodies, just as the excrements of higher organisms are injurious to the bodies from which they spring. Thus, if the excrements are allowed to remain in contact with the organism, they act as antiseptics.

MOVEMENT.— Some bacteria possess the faculty of spontaeous movement; they produce movement by wave-like motion of little thread-like projections of their bodies known as flagella or cilia; they can move independently only in liquids.

REPRODUCTION.— It is to their rapid reproduction that bacteria owe their wonderful influence in nature. They reproduce by simple division of the parent cell. The rate of multiplication depends on the food supply, environment, and species. Some of them, under favorable conditions, divide, thus doubling their number, in twenty minutes; others in three or four hours. Another method of reproducing or propagating is by spores. Spores may be likened to seeds of higher plants, although they are not identical. The spore is very resistant to external injurious influences. Disinfectants of sufficient strength to destroy bacteria are usually not destructive to spores. Spores require a much higher temperature for their destruction than do bacteria. Disinfectants should be made of sufficient strength to destroy spores; then the bacteria will also be destroyed.

WHERE FOUND.— Bacteria are omnipresent. They are in the air we breathe; in the crust of the earth; on trees, grass, hay, flowers, fruit, vegetables; in lakes, creeks, rivers, oceans, the water we drink, in our food—in fact, they are everywhere, except in the interior of the earth and the upper layers of the atmosphere.

NECESSARY CONDITIONS FOR BACTERIAL GROWTH. 1.— *Moisture.* A certain amount of moisture is necessary for all life. Drying is resorted to in the preservation of fruits, vegetables, meat, and even dried eggs are now on sale for food. The seeds of nearly all plants are preserved because of the lack of moisture in sufficient quantity to support bacterial growth. Hay and grains are preserved indefinitely if thoroughly dried before stacking or mowing.

2.— *Temperature.* The temperature requirements vary according to the species or variety. In general, disease-producing bacteria require the temperature of their host; fermentation bacteria, the ordinary summer temperature; some sea bacteria are quite active at the freezing temperature, while some soil bacteria thrive almost at the boiling temperature. The temperature range is very wide, and consequently we find bacteria widely distributed in nature. By controlling the temperature, we can, in many cases, govern bacterial growth. Thus foods are preserved by cold storage, the materials being actually frozen, and in this condition may be preserved almost indefinitely. But cold does not destroy bacteria; it only checks their action; hence the foods, if infected, will be acted upon immediately after removing from the cold storage. Even a temperature slightly above freezing is beneficial in preventing fermentation. Milk sours in a few hours at summer temperature, but if placed in spring- or well-water in cans or jars it will remain sweet for several hours, because the cooling lowers the temperature of the milk below the growing temperature of the bacteria that sour it. Hermetically sealed products are usually freed from bacteria by first heating the substance and then sealing in a sterile vessel. High temperature destroys bacteria; hence the value of cooling foods. City dairies usually sterilize the milk before starting on their route. Sterilizing kills the bacteria; hence the milk keeps sweet much longer.

3. *Absence of Sunlight.* Ordinary daylight is not, as a rule, very injurious to bacteria, but sunlight has a decidly injurious effect. Test experiments prove that two hours' exposure to sunlight will kill most bacteria. Modern buildings should have more light.

Bacteria also require certain mineral foods and certain respiratory gases. With a knowledge of the essential conditions for bacterial growth, man can largely control the development of them.

CLASSIFICATION .— There is considerable confusion in the classification of bacteria. There are four classes, according to their form: cocci or spherical, bacilli or cylindrical, spirilla or spirals, and the branching forms; and there are many classes, according to their bacteriological characteristics. Probably the most common classification

used is the pathogenic or disease-producing, and the non-pathogenic or those not producing disease.

METHODS OF STUDYING BACTERIA.—It may be of interest to state briefly the methods used in studying bacteria.

Gross examination: The gross examination is the study of the colony. A colony is a term applied to a mass or a group of bacteria developed from a single point (fig. 1), and preferably from a single bacterium; the colonies are grown on artificial food prepared from nutrient substances, such as beef or chicken broth or infusion of vegetable substance; the food may be used as a liquid, or it may be solidified by the use of gelatine or gummy substances. After the food is prepared and heated to destroy all bacteria in it, it is placed in sterile vessels for future use. It is on this prepared food that the bacteria are planted and allowed to develop, thus forming colonies. The colonies developed from different bacteria usually differ in some respects, thus giving a clue to the species or variety forming the colony.

STUDY OF THE INDIVIDUAL.—The individuals are studied by the use of the compound microscope, and are thus magnified sufficiently to study their structure. By the use of analine dyes they are stained, different species differing in their staining characteristics.

Specific Reactions.— The tests for specific reactions depend upon the knowledge of the bacteria in question. If practically nothing is known of its source, it is tested on animals and on various food materials, such as milk, potatoes, beef broth, etc.

WHAT BACTERIA DO.—The general function of bacteria is the reduction of complex organic compounds to simple substances. Plant life is built up from the mineral substances in the soil and atmosphere, at the expense of solar energy. Animals obtain their food either directly or indirectly from plants. When life ceases, either in plants or animals, bacteria disintegrate the dead bodies, returning the composing elements to the soil or atmosphere, to be again used in the construction of new forms of plant life. Thus the life cycle continues. "All that has lived must die, and all that is dead must be disintegrated—the elements which are the substratum of life must enter into new cycles of life." If the destructive bacteria should cease their work, the surface of the earth would be covered in a few years with mummies, unless cremation was universal. The law of perpetuity of life depends upon the use of food elements over and over again. The storage of food elements in the form of mummies year after year would in time exhaust the supply, and life would become extinct. Although the general function of bacteria is destructive, there is a group that is constructive, building up simple substances into more complex compounds.

FERMENTATION.–Fermentation is the decomposition of a substance into new substances by the action of a ferment. Ferments are produced by living cells and possess the following properties:

1. They are very sensitive to temperature. When frozen they are inactive, although they are not destroyed. Boiling destroys them.

2. A very small amount will change a large amount of material acted upon and remain unchanged itself.

3. Their action is checked or stopped completely by the accumulation of their products.

Fermentation includes practically all bacterial action. The production of disease by bacteria is now considered as typical a fermentation as is the decomposition of dead matter. The relation of soil bacteria to the production of plant food is of intense importance to the agriculturalist. It is to this latter phase of fermentation that we will now turn our attention.

Some of the following conclusions may be erroneous because of the short time work has been carried on, and the right is reserved to modify statements given out in this preliminary publication.

In the soil experiments, the primary tests have been on samples taken from the first thirty inches of soil from five different fields. All the samples were taken from as near the same place in the field as possible. The crop and tillage records of the five fields are shown herewith. The thirty inches were taken in four separate samples, one sample being taken each week. The first sample was the first eight inches of the soil; the second, the next seven inches, or nine to fifteen inches, inclusive; the third, the next eight inches, or sixteen to twenty-three inches, inclusive; and the fourth, the last seven inches, or twenty-four to thirty inches, inclusive.

Field No. 1.

Year	Method of cultivation.	Time of cultivation.	Time seeded.	Crops,	Remarks.
1888	Plowed.	{ Fall, '87. Spring, '88	May.	Corn.	
1889	"	Nov., '88.	"	Sorghum, ensilage.	
1890	"	March,	March.	Oats.	
1891	"	July, '90.	Sept.	Wheat.	
1892	"	Fall, '91.	May.	Sorghum, Kafir-corn.	
1893	———	———	———	———	
1894	Plowed.	Fall, '93.	May.	Soy-beans, cow-peas.	
1895	"	June.	June.	Navy-beans.	
1896	"	{ Spring, '96. Fall '95.	April.	Corn.	
1897	"	Nov., '96.	"	Alfalfa.	Still in alfalfa, 1902.

Field No. 1 has been cultivated since 1886.

Field No. 2.

Year.	Method of cultivation.	Time of cultivation.	Time seeded.	Crops.	Remarks.
1889	Plowed.	Aug., '88.	Sept., '88.	Wheat.	
1890	"	———	May 9.	Forage-plants.	
1891	"	Fall, '90.	April.	Forage-plants; grasses, variety.	
1892	"	" '91.	"	Corn.	Grasses not yet plowed.
1893	Plowed and disked	Spring.	March.	Oats.	
1894	Plowed.	Aug., '93.	Sept., '93.	Wheat.	Heavily manured this winter.
1895	"	Spring.	———	Ensilage, corn.	
1896	"	Fall, '95.	March.	Oats.	
1897	"	Aug., '96.	Sept, '96. May, '97.	Wheat. Corn, Kafir-corn.	Pastured and winter-killed, and other crop put in.
1898	"	March.	April.	Alfalfa.	

Field No. 2 has been in cultivation since 1886.

Field No. 3.

Year.	Method of cultivation.	Time of cultivation.	Time seeded.	Crops.	Remarks.
1888	Plowed.	Fall.	Oct. 1.	Wheat.	Plowed up May 10 to kill bugs.
1888	Part plowed and part not plowed.	Spring.	April 21. " 11.	Corn. Oats.	
1889	Spring-tooth harrow.	Fall. Spring.	May 11. March 20.	Millet. Oats.	
1890	Plowed.	Fall.	April 20. May 1,2,5,14.	Corn. Oats, millet.	
1891	"	"	April 8-10.	Barely. Oats.	
1892	"	"	Oct. 14.	Wheat.	
1893	"	———	Sept. 30. May 17.	Soy-beans. Cow-peas.	
1894	"	———	March 16. " 19.	Barley and oats. Oats.	
1895	"	Spring.	April 29. " 21.	Sunflowers. Corn.	
1896	"	Fall. Spring.	March 31. May 17.	Field peas. Kafir-corn.	
1897	"	Fall.	March.	Oats.	
1898	"	Spring, '97.	March. May.	Alfalfa. Kafir-Corn and sorghum	
1899	"	Spring, '98.	May.	Soy-beans.	Soil inoculated.
1900	"	Spring, '00.	April.	Mixed grass.	

Field No. 3 has been under cultivation since 1872.

Field No. 4.

Year.	Method of cultivation.	Time of cultivation.	Time seeded.	Crops.	Remarks.
1888	Plowed.	Fall, '87.	April.	Variety of grasses.	
1892	"	" '87.	——	——	
1893	Disked.	Spring.	April.	Corn.	
1894	Plowed. Disked.	Fall.	March. Sept.	Oats. Wheat.	
1895	Plowed. Disked.	Spring.	——	Field peas.	
1896	Plowed.	Fall.	Sept.	Wheat.	
1897	"	"	"	"	
1898	——	——	——	——	
1899	Plowed.	Fall.	April.	Corn.	
1900	"	May 29.	May.	Beans.	Inoculated field.
1901	Plowed Disked.	Spring. Aug.	Aug.	Field peas and oats. Rape.	
1902	Plowed.	Spring.	April.	Oats and wheat.	

Field No. 5.

Year.	Method of cultivation.	Time of cultivation,	Time seeded.	Crops.	Remarks.
1888	Plowed.	Fall.	Sept.	Rye.	
1889	"	Spring.	April.	East half, sorghum. West half, corn.	
1890	"	Fall, '89.	"	Corn.	
1891	"	" '90.	"	Oats, varieties.	
1892	"	Aug., '91.	Oct., '91.	Wheat.	
1893	"	Fall, '92.	April.	Corn.	
1894	"	" '93.	March.	Oats.	
1895	" Subsoiled.	Aug., '94. May, '95.	Sept. May.	Wheat. Corn.	Wheat winter-killed, then planted to corn.
1896	Double-listed and subsoiled.	Spring.	April.	Corn.	
1897	Plowed.	Dec., '96.	"	Oats.	
1898	Plowed.	Spring.	May.	Corn and Kafir-corn.	
1899	Plowed and packed.	"	"	Soy-beans.	
1900	Plowed.	"	April.	*Bromis inermis* alfalfa.	
1901	"	"	——	Corn.	
*1902					

*Alfalfa plat planted in spring.

SAMPLING.— It is difficult to obtain a pure sample of a particular portion of soil. Many methods have been used, but they all have some objections. The following method is simple, and it is as efficient as any. It is, briefly, as follows: Trenches were dug with an ordinary spade one foot wide and three feet long, the depth depending on the sample taken. One side of the trench was made as nearly perpendicular as possible, and the separate inches were marked off with a measuring-stick. Three samples were taken from each inch of soil with a sterile steel spatula and placed in one-half-ounce sterile, ground-glass-stopper bottles. The samples of one field were taken to the laboratory immediately after collecting, and plate cultures made of them, as follows: The soil sample in each sample bottle was thoroughly mixed; one decigram of the soil sample was then weighed on a beam-balance that was sensitive to one centigram; the decigram of soil was ground up in a sterile porcelain mortar with ten cubic centimeters of sterile water; one c.c. of this soil-water mixture was drawn off with a sterile pipette and placed in a sterile Phoenix graduate and diluted to 100 c.c., with sterile water; the solution in the Phoenix graduate was thoroughly mixed by pouring it back and forth into a sterile jar. One c.c. of the latter mixture was drawn off with a sterile pipette and placed in a tube of liquefied, sterile culture medium, with which it was thoroughly mixed before making the plate culture. Three plates were thus made from each inch sample; the amount of soil, dilution and the process being the same in each sample above described.

STERILIZING APPARATUS.— Because of the omnipresence of bacteria, sterile apparatus is absolutely essential for bacteriological analysis. The apparatus in the soil analysis were sterilized as follows: The steel spatula was sterilized before taking each sample by dipping it in a five-per-cent. solution of carbolic acid, then wiping it off with a cloth. The sample bottles and plates were thoroughly washed after using, and sterilized in a hot-air sterilizer at a temperature of from 140 degrees to 160 degrees C., for from three to four hours. The sample was taken from the sample bottles with a steel spatula, sterilized as above described, and weighed on clean paper, each paper being used for weighing one sample only. The mortar and pestle, pipettes, graduates and jars were sterilized by first rinsing thoroughly in a solution of hydrochloric acid, then in a one-per-cent. solution of sodium carbonate, and finally by rinsing three times in sterile water. The culture medium was sterilized in a steam sterilizer at steam heat for from three to four hours.

CULTURE MEDIUM USED.— An inorganic culture medium was used, composed of magnesium sulphate 0.1 gram, acid potassium phosphate

2 grams, normal ammonium phosphate 10 grams, sterile water 1000 grams, with 1 to 1.5 grams of agar to solidify it. Gelatin was tested, but many of the plates were liquefied before all the colonies were developed sufficiently to count. This medium was thoroughly tested with all common bacteria in 1901 by E. W. Doane. The inorganic medium has the advantage of being more transparent than the organic.

Moisture was determined by placing a known quantity of soil remaining after sampling in square tin boxes and drying in an oven at a temperature of from 90 degrees to 95 degrees C. The dried soil was then weighed and the correction made.

COUNTING.— The plates were placed on a table in the laboratory for from three to five days, or until the colonies were developed. The temperature of the laboratory varied from 20 degrees to 30 degrees C. There may be as many as 10,000 colonies in a plate culture; hence it is impracticable to count all of them. The sample was thoroughly mixed with the culture medium before making the plate culture; consequently the bacteria were equally distributed, and hence equal areas of the plate contained practically the same number of colonies. A glass plate ruled in squares was used for counting (fig. 2). The area of each square in the counter was 0.01 of a square inch. Two squares in three different places on the counter were inked (fig. 2), and the colonies were always counted in these squares. The ruled glass plate was placed on the bottom of the culture and twenty of the squares were counted, as shown in fig. 3, six squares being counted in one, six in two, six in three, and two in four. The counting points were always taken without any selection. The twenty squares are equivalent to one-fifth of a square inch. The number of colonies in the whole plate culture was estimated from the number of squares in the plate and multiplying by the average number of bacteria in one square of the twenty squares counted.

1. *Influence of Kind of Soil.*— The average number of bacteria for one gram of soil from the thirty inches of each of the five fields for May, June and July is shown in the following table:

TABLE I.

Field 1	1,017,954,242.
" 2	1,618,681,810.
" 3	2,356,025.
" 4	289,290,181.
" 5	105,763,909.

This difference of bacterial content cannot be attributed to the difference in tilling and cropping, neither could it be due to the soil moisture, because the percentage of soil moisture in the different

FIG. 1. Plate Culture, showing Colonies.

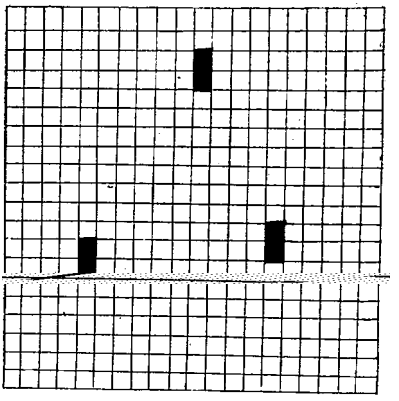

FIG. 2. Counting plate.

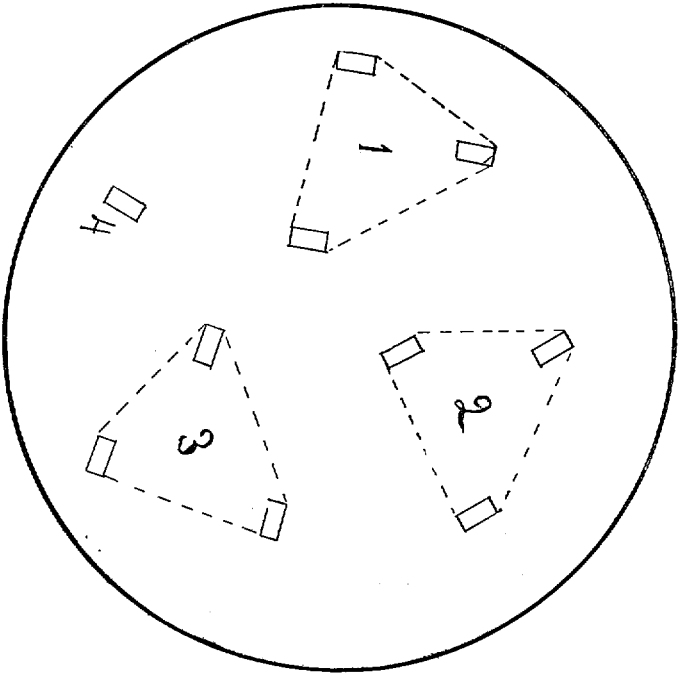

FIG. 3. Position of counting plate for counting colonies.

fields did not vary more than two per cent. But the soil is not the same in all fields. Field 1 is a black loam containing considerable humus. Field 2 is similar to field 1, but contains more humus. Field 3 is a thin soil with a clayey gumbo subsoil. Fields 4 and 5 are black loam, but not as rich in humus as either field 1 or field 2.

The crop records of these fields for the past ten years indicate that the crop yield has been directly proportional to the bacterial content of the soil of each field; that is, field 2 has produced the largest yield, and field 3 has produced the least.

Although the productiveness of the soil is largely dependent upon the humus it contains, this is not the only factor. Humus is a favorable medium for bacterial development; hence the bacterial content is not a direct index to the productiveness of the soil. Cultivation is an important factor in crop production.

2.— *Influence of the Crops.* The following samples were all taken near the College and are a composite from the first twelve inches of soil:

TABLE II.

Crop.	Bacteria per gram.
Oats	809,400
Kafir-corn.	3,186,000
Corn	1,473,400
Alfalfa	1,287,000
Soy-beans	3,894,000
Prairie bluestem.	622,300
Timber.	638,000

The soil in all the above fields is similar. This being a single test, it should not be conclusive.

3.— *Influence of Depth.* The greatest number of bacteria is not found at the surface, but some distance below—usually from about the tenth to the fifteenth inch. Kramer found that the bacteria decreased gradually from the surface down, as is shown in the following table:

Depth.	Bacteria per gram.
8 inches	650,000
19 "	500,000
27 "	276,000
39 "	36,000
47 "	5,600
55 "	700
65 "	practically no bacteria found.

Housson found rather a gradual decrease down to about the fourth foot, as is shown by the following:

	Bacteria per gram.
At the surface.	1,680,000
At a depth of two feet. . . .	900,000
At a depth of four feet. . . .	25,000
At a depth of six feet.	410

The following table is an average of the number of bacteria in one gram of soil for each of the thirty inches in the five experimental fields:

TABLE III.

Inch.		Inch.	
1	11,639,000	16	7,579,613
2	44,961,959	17	6,144,393
3	39,753,826	18	6,688,080
4	41,024,040	19	5,185,906
5	75,517,306	20	5,278,800
6	89,089,773	21	8,425,120
7	92,656,713	22	8,281,333
8	165,260,380	23	4,256,630
9	116,801,020	24	5,037,550
10	97,574,253	25	14,189,330
11	44,737,026	26	13,790,330
12	26,388,200	27	18,281,280
13	16,898,540	28	11,467,200
14	19,788,426	29	15,639,980
15	13,610,113	30	12,552,700

To obtain the average given in table III, one sample was taken each month for May, June, and July, from each inch of soil, and three plates made from each sample, making a total of forty-five plates from each inch upon which to base the estimate. As the table indicates, there is a gradual increase from the surface down to the eighth inch; then the number diminishes down to the nineteenth or twentieth inch, after which there is a slight increase, and a final decrease down to the thirtieth inch.

4. *Influence of the Time of Year.*— Since the experiments have extended over only three months of time, the results are open to objection, and no conclusion can be drawn as to the variation throughout the year. The following table shows the average number of bacteria for one gram of soil from each of five fields through May, June, and July:

TABLE IV.

May	528,746,000
June	5,748,666
July	8,729,600

Each of the above sums is an average of the results from 450 plate cultures.

5.— *Influence of Location.*— By the aid of some of the institute workers, samples were collected from various locations in the state. The samples were collected in bottles the same as those described above. They were expressed to the laboratory in boxes made from two-inch material, by boring holes the same diameter and as deep as the bottles were high, then screwing on thin strips of board over them. Each sample box contained four bottles. The following directions accompanied each sample box:

DIRECTIONS FOR SOIL-SAMPLING.— 1. Obtain one sample of prairie soil and two of cultivated soil. Of the samples from the cultivated soil, take one from wheat, corn, or Kafir-corn; and one from alfalfa, soy-beans, or peas.

2. Dig a trench with a clean spade one foot wide, one foot deep, and three feet long. Make one side as nearly perpendicular as possible.

3. Take from the perpendicular side a small sample with a clean pocket-knife, from three different points in each of the twelve inches. Do not fill sample bottle more than three-fourths full.

4. Screw top down, and express, C. O. D., veterinary department, as soon as convenient.

THE RESULTS.—The following table gives the number of bacteria per gram from a composite sample of the first foot of soil.

TABLE V.

Collected.	Kind of soil.	Crop.	County.	Collector.	Bacteria per gram.
Aug. 15	Black loam	Timothy and clover	Marshall	A. T. Kinsley	1,380,000
"	"	Alfalfa and clover	"	"	21,091,000
"	"	Corn	"	"	994,500
"	"	"	"	"	2,035,825
"	"	Prairie bluestem	"	"	778,800
"	"	"	"	"	718,750
Aug. 20	"	Alfalfa	Cloud	R. F. Bourne	1,872,500
"	"	Buffalo-grass	"	"	2,247,200
"	Sandy	Wheat	"	"	1,346,200
Aug. 21	Black loam	Melons	"	"	2,177,400
Aug. 22	Upland flat	Buffalo-grass	Grant	E. A. Popenoe	1,451,500
"	Bottom	Sorghum	"	"	897,120
Aug. 25	Upland	"	Scott	D. E. Lantz	201,400
"	Black loam	Alfalfa	"	"	885,500
"	Gumbo	Uncultivated	"	"	272,500
"	Black loam	Cow-peas	"	"	352,000
"	"	Irrigated garden	Greeley	"	168,000
"	Light loam	Gramma-grass	"	"	357,000
"	Black loam	Corn	"	"	256,000
Aug. 28	"	Alfalfa	Mitchell	A. T. Kinsley	573,400
"	"	Corn	"	"	266,800
"	"	Prairie buffalo-grass	"	"	324,800
Sept. 1	Light loam	Prairie buffalo-grass	Logan	"	888,000
"	"	Irrigated garden	"	"	545,200
"	"	Corn	"	"	699,300
Sept. 2	Black loam	"	Ellis	"	422,400
"	"	Prairie buffalo-grass	"	"	725,400
"	Sandy loam	Prairie buffalo-grass	"	"	143,000
"	"	Alfalfa, 2d year	"	"	278,400
"	"	" 1st year	"	"	443,000
"	"	" several yrs.	"	"	
"	Black loam	Soy-beans	"	"	394,400

The above tests are of interest because of the high bacterial content of soil containing buffalo-grass. It also shows a marked decrease in the bacterial content in the western part of the state.

Owing to the limited time of the experiment, the primary test was for numbers; however, the following-named bacteria have been isolated: the scheme followed is a modification of the scheme used by Doctor Novy in his Laboratory Guide:

Bacillus subtilis, hay bacillus.
Origin.— Air, water, soil, excretions, putrefactive products, etc.
Form. — Cylindrical.
Motility. — Very actively motile.
Sporulation. — Forms highly resistant spores.
Temperature. — Minimum, 10°; maximum, 45°; optimum, about 30°.
Function. — Produces general putrefaction.

Bacillus mesentericus vulgatus, potato bacillus.
Origin. — Same as hay bacillus.
Form. — Cylindrical.
Motility. — Very motile.
Sporulation. — Forms oval spores.
Temperature. — Optimum, about 30°; this organism is active at a much higher temperature than the hay bacillus.
Function. — Produces sweet curd in milk, and is one of the general putrefactive organisms.

Bacillus megaterium.
Origin. — Same as hay bacillus.
Form. — Usually cylindrical, but is variable in form according to environment.
Motility. — Slightly motile.
Sporulation. — It forms large central spores.
Temperature. — Same as the hay bacillus.
Function. — This organism was first found on cabbage and was thought to have some functions concerning the ripening of cabbage. It is a putrefactive organism.

Bacillus kralii.
Origin. — Soil, air, and water.
Form. — Cylindrical.
Motility. — Not motile.
Sporulation. — No spores observed.
Temperature. — 25° to 30°.
Function. — Putrefaction.

Bacillus epsilon.
Origin. — Air and soil.
Form. — Cylindrical.
Motility. — Slightly motile.
Sporulation. — Forms no spores.
Temperature. — 25° to 30°.
Function. — Putrefaction.

Bacillus helvolus; Bacillus chromo-aromaticus.
Origin. — Soil,
Form. — Cylindrical.
Motility. — Not motile.
Sporulation. — No spores.
Temperature. — 20° to 25°.
Function. — Putrefaction, and pathogenic in rabbits.

Bacillus mycoides.
Origin. — Soil, air, water, etc.
Form. — Cylindrical.
Motility. — Slightly motile.
Sporulation. — Forms no spores.
Temperature. — 20° to 30°.
Function. — Putrefaction.

Bacillus liquifaciens.
Origin. — Air, water, soil, animal body excretions, etc.
Form. — Cylindrical.
Motility. — Motile.
Sporulation. — Does not form spores.
Temperature. — 20° to 30°.
Function. — Putrefaction.

Bacillus glaucum.
Origin. — Milk, air, soil, and water.
Form. — Cylindrical.
Motility.— Motile.
Sporulation. — No spores observed.
Function. — Putrefaction.

Micrococcus rugosus.
Origin. — Soil, water, air, milk, etc.
Form. — Spherical.
Motility. — Not motile.
Sporulation. — Does not form spores.
Temperature. — 28° to 30° C.
Function. — Putrefaction.

Bacillus aurantiacus.
Origin. — Air, water, and soil.
Form. — Spherical.
Motility. — Not motile.
Sporulation. — Does not form spores.
Temperature. — 25° to 30° C.
Function. — Putrefaction.

Micrococci acidi lactici.
Origin. — Air, water, soil, and milk.
Form. — Spherical.
Motility. — Not motile.
Sporulation. — Does not form spores.
Temperature. — 250 to 35° C.
Function. — Converts lactose into lactic acid.

Staphylococcus cereus flavus.
Origin. — Air, soil, water, etc.
Form. — Spherical, grouped in masses.
Motility. — Not motile.
Sporulation. — Does not form spores.
Temperature. — 25° to 35° C.
Function. — Decomposition.

Staphylococcus pyogenes citreus.
Origin. — Air, soil, water, carbuncles, etc.
Form. — Spherical, grouped in masses.
Motility. — Not motile.
Sporulation. — Does not form spores.
Temperature. — 30° to 35° C.
Function. — Pyogenic and decomposition.

Streptococcus ochroleucus.
Origin. — Animal excrements, air, soil, water.
Form. — Spherical.
Motility. — Not motile.
Sporulation. — Does not form spores.
Function. — General decomposition.

Micrococcus agilis citreus.
Origin. — Air, soil, and water.
Form. — Cylindrical.
Motility. — Actively motile.
Sporulation. — No spores observed.
Function. — Putrefaction.

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It is hoped that the soil experiment will be continued, as it is impossible to generalize conclusions from the work done.