

## QUANTITATIVE AND QUALITATIVE BACTERIAL ANALYSIS OF SOIL SAMPLES TAKEN IN FALL OF 1918

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A bacterial analysis was made of 46 soils representing 17 soil types obtained in a soil survey of Russell, Carleton and Stormont counties in Eastern Ontario. Each soil was represented by two samples, one taken from the surface to a depth of  $6\frac{3}{4}$  inches, and the other a sub-surface sample taken from  $6\frac{3}{4}$  to 20 inches depth. The lower subsoils were not examined for bacterial content. These soils were filled directly into cotton bags in the field where obtained and shipped to the college by freight. On arrival at the chemistry department they were emptied into large open-topped dishes for air-drying in the attic, which was clean and free from dust. The samples for bacterial analysis were taken from these open dishes a few days after their arrival and put directly into clean, dry 500-cc. Erlenmeyer flasks which were half filled and then corked and kept at a low temperature in the attic of the bacteriology department until December 15, when the analyses were begun.

The purpose of the analysis was to determine the relative bacterial and mold content of the different soil samples. Three culture media were used as follows:

1. *Albumen Synthetic Agar* (A) (P. E. Brown) for obtaining the total bacterial and mold counts.
2. *Nutrient Gelatin* for obtaining the liquefier counts.
3. *Modified Ashby's Agar*<sup>1</sup> for obtaining the *Azotobacter*, *Ps. radiculicola* and *Nocardia* counts.

### SOIL DILUTIONS

After thoroughly mixing the soil samples and discarding any gravel or lumps, 20 gm. of the remaining soil were weighed out on a piece of flamed copperfoil, and put into a 500-cc. Erlenmeyer flask containing 200 cc. of sterile water.

This was shaken for 5 minutes and after the coarser particles had settled, 10 cc. of the mixture was transferred to a 90-cc. sterile water blank which was thoroughly shaken, and from this 10 cc. was transferred to another 90-cc. water blank and further dilutions were made in the same way until a series of 1/10, 1/100, 1/1,000, 1/10,000, 1/100,000 and 1/1,000,000 gram of soil

<sup>1</sup> Ashby's agar was modified by using 10 gm. of cane sugar in place of 20 gm. of mannite.

per cubic centimeter were obtained. In general the higher dilutions were used for the total counts of the surface soils, and the lower dilutions for the subsoils.

The prevailing types of colonies were isolated from the various plate cultures on the tubes of similar medium to that of the plate from which they were taken. These tube cultures were then used for making microscopic preparations for describing the organisms.

The counts recorded are the average counts from plate cultures made from three dilutions of each soil sample.

The *Ps. radicola* counts are only approximate, as the colony appearance of some other species of bacteria rather closely resembles that of *Ps. radicola*.

TABLE 1  
*Soil type I—Yellow sand*

SAMPLE NUMBER	DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICOLA	NOCARDIA	MOLDS
Sur. 1	Typical	373,000	413,000	0	0	20,000	120,000
Sub. 2		433,000	403,000	0	0	30,000	65,000
Sur. 4	Finer sand, similar to type 2	380,000	10,000	0	0	100,000	140,000
Sub. 5		340,000	2,000	0	0	400,000	65,000

TABLE 2  
*Soil type 2—Light to medium brown, fine sand and sandy loam*

SAMPLE NUMBER	DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICOLA	NOCARDIA	MOLDS
Sur. 7	Representing the fine sand. Clay subsoil	910,000	56,000	0	0	300,000	190,000
Sub. 8		950,000	1,000	0	0	100,000	100,000
Sur. 10	Same field as 7-8 but taken from hollows	273,000	24,000	0	0	200,000	5,500
Sub. 11		200,000	2,000	0	0	0	10,000
Sur. 13	Shallow, not so sandy. Clay subsoil of 13	740,000	77,000	0	100,000	300,000	500,000
Sub. 14		1,300,000	5,000	0	100,000	200,000	130,000
Sur. 17	Representing the fine sandy loam	810,000	46,000	0	200,000	600,000	80,000
Sub. 18		270,000	8,000	0	0	0	60,000
Sur. 20	Typical. Same as 17-18	140,000	19,000	0	70,000	240,000	10,000
Sub. 21		433,000	8,000	0	30,000	80,000	25,000
Sur. 23	Same as 13-14 but clay subsoil of 8	2,150,000	100,000	0	0	200,000	670,000
Sub. 24		1,880,000	15,000	0	400,000	0	160,000

TABLE 3

*Soil type 3—Light to medium brown, gravelly, sandy loam*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	26	Typical	2,650,000	105,000	195	900,000	600,000	20,000
Sub.	27		3,350,000	27,000	75	1,200,000	800,000	185,000
Sur.	29	Flat, sand	2,270,000	240,000	40	500,000	200,000	130,000
Sub.	30		1,110,000	38,000	20	700,000	200,000	60,000
Sur.	32	Typical	1,750,000	91,000	0	200,000	100,000	1,200,000
Sub.	33		1,000,000	54,000	0	200,000	100,000	470,000
Sur.	35	Typical	1,440,000	41,000	10	200,000	400,000	260,000
Sub.	36		650,000	13,000	0	0	200,000	320,000

TABLE 4

*Soil type 4—Medium brown loam, in which stones vary in size and quantity. Variable gravel content*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	38	Typical of medium soil	1,730,000	12,000	1,710	600,000	1,400,000	40,000
Sub.	39		1,110,000	55,000	40	100,000	200,000	100,000
Sur.	41	Less stone and gravel in surface, more clay in subsoil	3,780,000	30,500	230	1,000,000	700,000	0
Sub.	42		2,980,000	210,000	0	200,000	600,000	10,000
Sur.	44	Probably belongs to type 3	950,000	355,000	0	100,000	200,000	0
Sub.	45		1,020,000	25,500	0	600,000	300,000	0
Sur.	47	Shallow soil, hard-pan or boulder, clay subsoil	1,030,000	64,500	170	400,000	800,000	30,000
Sub.	48		513,000	64,500	120	0	100,000	80,000
Sur.	49	About the same as 38-39	3,270,000	66,000	520	400,000	300,000	200,000
Sub.	50		1,700,000	79,000	80	1,200,000	200,000	240,000
Sur.	52	Slightly more gravel than 49-50 and 38-39	3,300,000	155,000	345	400,000	900,000	550,000
Sub.	53		2,330,000	95,000	70	300,000	200,000	295,000

TABLE 5  
*Soil type 5—Black sandy loam*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	55	Typical	3,970,000	260,000	410	200,000	1,300,000	140,000
Sub.	56		255,000	125,000	25	800,000	200,000	5,000
Sur.	58	Lighter in color than typical soil	4,770,000	93,000	210	400,000	200,000	340,000
Sub.	59		610,000	55,000	0	200,000	0	55,000
Sur.	61	Typical	1,610,000	63,000	10	0	400,000	65,000
Sub.	62		1,320,000	8,500	10	200,000	0	22,000
Sur.	64	Same area as 58-59, practically belongs to type 2	2,230,000	53,000	0	0	100,000	21,500
Sub.	65		540,000	8,500	0	0	0	65,000

TABLE 6  
*Soil type 6—Sandy clay loam, usually dark brown*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	67	Typical except that the sand is of finer grade than usual	2,870,000	61,000	660	1,900,000	300,000	10,000
Sub.	68		1,830,000	55,000	80	1,600,000	0	0
Sur.	70	Probably belongs to type 8	4,100,000	160,000	85	800,000	400,000	40,000
Sub.	71		650,000	15,500	0	400,000	0	20,000
Sur.	73	Typical	3,750,000	190,000	1,800	T.M.	T.M.	0
Sub.	74		3,250,000	185,000	125	T.M.	T.M.	0

TABLE 7  
*Soil type 7—Brown, and usually moderately stony, clay loam*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	76	Lighter than typical soil	3,700,000	170,000	970	700,000	1,000,000	170,000
Sub.	77		2,500,000	226,000	850	800,000	600,000	75,000
Sur.	79	Heavier than typical soil	9,100,000	68,000	60	5,000,000	700,000	230,000
Sub.	80		4,600,000	67,000	0	3,500,000	200,000	260,000
Sur.	82	Typical	4,400,000	750,000	120	1,000,000	1,500,000	20,000
Sub.	83		3,400,000	900,000	75	600,000	850,000	15,000
Sur.	85	Typical	8,500,000	122,000	0	500,000	1,900,000	245,000
Sub.	86		1,670,000	4,000	0	1,000,000	100,000	210,000

TABLE 8

*Soil type 8—Very fine, sandy, heavy clay loam and clay, usually dark gray to black*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	88	Typical	6,980,000	385,000	90	1,500,000	1,200,000	195,000
Sub.	89		666,000	20,500	0	400,000	100,000	65,000
Sur.	91	Surface a little lighter than 88-89	2,680,000	40,000	0	200,000	100,000	20,000
Sub.	92		1,020,000	20,500	0	300,000	0	0
Sur.	94	Typical, but not so old; higher nitrogen content	5,100,000	76,000	0	1,400,000	700,000	135,000
Sub.	95		690,000	4,000	0	0	0	30,000

TABLE 9

*Soil type 9—Muck and peat with clay subsoil*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	97	Typical	5,270,000	850,000	0	700,000	1,900,000	75,000
Sub.	98		880,000	28,500	0	90,000	70,000	200,000

TABLE 10

*Soil type 10—Very fine, sandy, silty loam*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	100		1,700,000	47,000	230	500,000	0	185,000
Sub.	101		155,000	12,000	0	300,000	100,000	150,000
Sur.	103	Typical	2,300,000	650,000	770	480,000	620,000	25,000
Sub.	104		1,060,000	390,000	200	960,000	350,000	30,000

TABLE 11

*Soil type 11—Silty loam*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	106	Typical	385,000	280,000	60	120,000	350,000	260,000
Sub.	107		640,000	60,500	0	220,000	200,000	75,000

TABLE 12

*Soil type 12—Red shale loam*

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	112	Typical	3,150,000	585,000	0	270,000	830,000	75,000
Sub.	113		3,900,000	250,000	0	450,000	T.M.	95,000

TABLE 13  
Soil type 13—Brownish red clay (shale)

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTO-BACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	114	An average sample of the large areas belonging to this type, though color not quite typical	1,780,000	26,500	0	300,000	700,000	30,000
Sub.	115		1,720,000	9,000	0	0	700,000	80,000
Sur.	117	Typical	9,800,000	155,000	0	T.M.	T.M.	250,000
Sub.	118		14,400,000	59,000	0	T.M.	T.M.	1,000

TABLE 14  
Soil type 14—Like type 5 in texture, but belongs to a shale series

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	120	Typical	4,200,000	105,000	0	700,000	T.M.	0
Sub.	121		5,300,000	280,000	0	800,000	T.M.	0

TABLE 15  
Soil type 15—Dark gray to black, shale clay

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	123	Typical	2,100,000	180,000	0	380,000	640,000	150,000
Sub.	124		4,100,000	38,000	0	160,000	730,000	10,000
Sur.	126	Also typical, but from an area that has not been cultivated so long as 123-124; more organic matter	4,180,000	93,000	0	0	700,000	230,000
Sub.	127		1,730,000	10,500	0	0	200,000	190,000

TABLE 16  
Soil type 16—Gray shale loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	129	A brown shale loam has more organic matter and is a good soil	4,830,000	435,000	0	1,000,000	800,000	120,000
Sub.	130		750,000	83,000	0	0	0	180,000
Sur.	132	Typical	3,000,000	57,000	0	580,000	400,000	200,000
Sub.	133		1,870,000	12,000	0	620,000	240,000	240,000

TABLE 17  
Soil type 17—Stony, fine sandy loam

SAMPLE NUMBER	DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFIERS	AZOTOBACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur. 135	Typical of best phase; inconveniently stony but passable as a soil	7,400,000	425,000	970	150,000	350,000	270,000
Sub. 136		4,200,000	50,000	295	420,000	190,000	320,000
Sur. 138	Bouldery and stony, only fit for pasture for sheep	3,500,000	47,000	0	900,000	600,000	315,000
Sub. 139		2,100,000	100,000	0	300,000	300,000	120,000

## DISCUSSION

*Relation of Azotobacter, the principal nitrogen-fixing species of soil bacteria, to the soil types*

It will be seen from the above counts that *Azotobacter* were found present in 9 out of the 17 soil types examined and in 22 out of the 29 soil samples that represented these 9 types. These types included all the loams except the shale loam as follows:

SOIL TYPE NUMBER	SOIL TYPE	PRESENCE OF AZOTOBACTER
3	Light brown gravelly sandy loam	3 out of 4 samples gave Azotobacter
4	Medium brown loam, stone varying in size and quantity	5 out of 6 samples gave Azotobacter
5	Black sandy loam, clay subsoil	3 out of 4 samples gave Azotobacter
6	Sandy clay loam	3 out of 3 samples gave Azotobacter
7	Brown, moderately strong clay loam	3 out of 4 samples gave Azotobacter
8	Fine sandy heavy clay loam	1 out of 3 samples gave Azotobacter
10	Fine sandy silty loam	1 out of 3 samples gave Azotobacter
11	Silty loam	1 out of 1 sample gave Azotobacter
17	Stony fine sandy loam	1 out of 2 samples gave Azotobacter

On the other hand, no *Azotobacter* were found in the remaining 8 soil types which included the sand, peat-muck and shale soils as follows:

SOIL TYPE NUMBER	SOIL TYPE	ABSENCE OF AZOTOBACTER
1	Yellow sand	2 samples
2	Light brown sand and sandy loam	6 samples
9	Muck and peat with clay subsoil	1 sample
12	Red shale loam	1 sample
13	Brownish red clay shale	2 samples
14	Brownish red clay shale, but sandy	1 sample
15	Dark gray to black shale clay	2 samples
16	Gray shale loam	2 samples

We assume from the above that in all probability the light sandy soils had not sufficient organic matter present and the peat-muck and shale soils were too strongly acid to favor the development of the *Azotobacter* nitrogen-fixing bacteria.

*Relation of Ps. radicola, the legume root nodule bacteria, to the soil types*

As the colony appearance of *Ps. radicola* is rather closely simulated by several other species of bacteria, we cannot be sure that all the counts above recorded as *radicola* are true members of this species, although every care was taken to discard any that were not true to type.

It is interesting to note that every soil type examined, with the exception of one only, i.e., type 1, yellow sand, had a fairly high *Ps. radicola* content, and further, that in most cases the sub-surface samples had a higher *radicola* content than the surface samples. This is as one might expect, as the *Ps. radicola* would come largely from the decayed legume root nodules.

In general, the soils that were favorable for *Azotobacter* had the higher *radicola* counts, but the fact that fairly high *radicola* counts were obtained in the peat-muck and shale samples, which were presumably too acid for *Azotobacter* would indicate that some varieties of *Ps. radicola* at least had a fair degree of toleration for acid soils.

We could not determine from a bacterial culture examination what different varieties of *Ps. radicola* were present, as the different varieties are determined by the different varieties of legume with which they will associate.

Therefore, we cannot say whether or not all varieties of legumes could be grown satisfactorily on all these different soils, even though *Ps. radicola* was found present in considerable numbers, as these numbers may represent only a limited number of legume varieties, thus limiting the choice of legumes to those varieties with which the varieties of *Ps. radicola* found would associate.

*Relation of the total bacterial count and liquefier count to the soil types*

In general the total bacterial count and liquefier count represent the majority of bacterial species that are active in digesting and preparing the crude plant-food present in the soil, thereby rendering it available in a suitable condition for assimilation by the growing crops. The number found is considered in a general way as being indicative of soil fertility, as their action is essential in the preparation of the plant-food in the soil.

With the exception of sample 1, yellow sand, sample 2, light brown sand, and sample 11, silty loam, each of which has a very low total count, all the samples have a fair bacterial content for arable soils. It is interesting to note that the shales have as high a total count as the loams; as a matter of fact one shale, no. 13, has the highest count of all. This would indicate that while the shale and peat muck soils examined were not suitable for the nitrogen-fixing *Azotobacter*, they were about equally suitable with the loams for the species of the plant-food digesting bacteria.



*Relation of Nocardia (Actinomycetes) count to the soil types*

It is considered (J. Conn) that the *Nocardia* (*Actinomycetes*) or thread bacteria, found in soil are largely active in bringing about the decay of dead root fibers, particularly those of sod, thereby aiding in preparing them for food of growing crops.

The counts above recorded show that they were least numerous in the sand but averaged up much alike in the loams, peat muck and shales, in all of which they were fairly numerous.

*Molds*

It is not at present considered that molds have any important rôle to play in connection with soil fertility. It is, however, interesting to note from the above counts that type 1, the yellow sand which gave no *Azotobacter*, no *radicicola* and the lowest of all total bacterial counts, gave a fairly high mold count, while type 6, sandy clay loam, which gave the highest *Azotobacter* count, a very high *radicicola* and total bacteria count, showed no molds. With one other exception, type 14, a sandy clay shale, which also gave no molds, the mold content of the samples was fairly uniform.

A chemical and physical analysis of these soils is being made, respectively, in the chemistry and physics departments of the college.