

SOIL BACTERIAL TYPES AND GREEN MANURING.

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In an experiment to determine the effect of calcium carbonate on the biological activities of a soil, it was noted that the addition of green manure seemed materially to alter the equilibrium existing between the bacterial types. This point was deemed of sufficient importance to justify further study and as a result an effort was made to classify the bacterial types developing on agar plates from the treated and untreated soils.

Many investigators have reported the effect of various green manures on the number of bacteria developing in the soil and practically all have found a decided increase following the addition of green manure. However, few investigators have attempted to make a qualitative study of this increase. Hiltner and Stömer¹, in Germany, and Chester² and Conn³, in this country, have carried out the most extensive work in studying and classifying the soil flora. Conn, whose work is far more thorough than any of the others, has classified the soil flora into three main divisions, spore formers, non-spore formers and Actinomycetes. In this same work, Conn reports that the addition of manure to soil disturbs the equilibrium between the bacterial types, greatly stimulating the non-spore forming group. Murray⁴, in 1920, reports that the addition of straw to the soil does not appear to stimulate any one type more than the others. Other investigators have reported similar results, the effect varying with the material applied to the soil.

The soil used in this experiment was a black sandy loam, containing a rather high percentage of organic matter and with a lime requirement of about 1,600 pounds calcium carbonate to the acre. Twenty gallon stone jars were filled with ten pounds each of the air dry soil and treated as follows:

- Pots 1 and 6 received 500 pounds calcium carbonate per acre.
- Pots 2 and 7 received 1,000 pounds calcium carbonate per acre.
- Pots 3 and 8 received 2,000 pounds calcium carbonate per acre.
- Pots 4 and 9 received 4,000 pounds calcium carbonate per acre.
- Pots 5 and 10 received no calcium carbonate.
- Pots 11 and 16 received 500 pounds calcium carbonate per acre.
- Pots 12 and 17 received 1,000 pounds calcium carbonate per acre.
- Pots 13 and 18 received 2,000 pounds calcium carbonate per acre.
- Pots 14 and 19 received 4,000 pounds calcium carbonate per acre.
- Pots 15 and 20 received no calcium carbonate.

¹ Hiltner, L., and Stömer, K. Studien über die Bakterienflora des Ackerbodens, mit besonderer Berücksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache. Kaiserl. Gesundheitsamte, Biol. Abt. Land- u. Forstw. 3, 445-545. 1903.

² Chester, F. D. Bacteriological analysis of soils. Del. Agr. Exp. Sta. Bul. 65. 1904.

³ Conn, H. J. Soil Flora Studies. New York (Geneva) Agr. Exp. Sta., Tech. Bul. 57, 58, 59, 60. 1917.

⁴ Murray, T. J. The effect of straw on the biological soil processes. Soil Science, 12, 233-259. 1921.

In addition to the above treatment, each pot from 1 to 10 inclusive, received green manure in the form of young rye plants, at the rate of twelve tons per acre.

These pots were placed in the rose house of the Purdue horticultural greenhouses and planted to soybeans. Moisture and temperature conditions were kept at optimum.

Bacterial numbers were determined by plating with standard beef extract agar and incubating fourteen days at room temperature, which was about 20° C.

The bacterial types were studied by sub-culturing on each of the following media:

1. Beef extract agar slants.
2. Beef extract bouillon.
3. Beef extract gelatin slabs.
4. Nitrate reduction solution.
5. Dextrose fermentation tubes.
6. Lactose fermentation tubes.
7. Brom cresol purple milk.

Each culture was also studied microscopically, noting shape, spore formation and motility.

TABLE 1. Bacterial Numbers. Millions per Gram of Soil.

Treatment		Dates of Sampling			
CaCO ₃ per acre	Green manure per acre	Oct. 19	Nov. 16	Dec. 14	Jan. 11
500 lbs	12 tons	1.2	20.0	4.2	7.0
1,000 lbs	12 tons	3.2	15.0	3.2	4.2
2,000 lbs	12 tons	3.5	10.5	5.7	4.5
4,000 lbs	12 tons	1.9	11.2	7.0	4.7
None	12 tons	0.8	9.2	3.9	4.7
Average		2.1	13.2	4.8	5.0
500 lbs	None	1.2	8.2	3.1	6.2
1,000 lbs	None	2.0	5.0	2.8	5.4
2,000 lbs	None	1.4	9.7	2.7	4.6
4,000 lbs	None	0.8	7.0	2.6	4.5
None	None	1.1	5.0	4.4	5.5
Average		1.3	7.0	3.1	5.2

Plate counts were made at four different times as shown in Table 1. While the applications of calcium carbonate exerted no appreciable effect on bacterial numbers, the application of green manure greatly increased the total count of bacteria. This is particularly true at the date of the second sampling, four weeks after the experiment was started, when decomposition of the green manure was progressing very

rapidly. At the date of the last sampling, three months after the experiment was started, practically all of the green manure had broken down and the bacterial counts were about the same as in those which had received no green manure.

At the date of the last sampling, ten colonies were picked from the agar plates of each treatment, making one hundred colonies, and were studied as previously described. Care was exercised to make as representative a selection as possible from each set of plates.

TABLE 2. Bacterial Types, as Influenced by CaCO₃ and Green Manure.

Treatment		Form			Motility		Sporeulation		Gelatin liquefaction	
CaCO ₃ per acre	Green Manure per acre	Coccus	Rod	Actinomyceete	+	-	+	-	+	-
500 lbs.	12 tons ..	2	6	2	5	5	6	4	9	1
1,000 lbs.	12 tons ..	3	6	1	5	5	1	9	9	1
2,000 lbs.	12 tons ..	3	7	0	2	8	2	8	10	0
4,000 lbs.	12 tons ..	4	6	0	6	4	2	8	7	3
None	12 tons ..	3	6	1	3	7	5	5	8	2
Total		15	31	4	21	29	16	34	43	7
500 lbs.	None	3	4	3	2	8	5	5	7	3
1,000 lbs.	None	6	4	0	3	7	3	7	7	3
2,000 lbs.	None	3	5	2	5	5	5	5	9	1
4,000 lbs.	None	4	5	1	5	5	5	5	9	1
None	None	7	3	0	3	7	2	8	7	3
Total		23	21	6	18	32	20	30	39	11

Table 2 gives a summary of this work. As in the case of the total bacterial counts, it is seen that the calcium carbonate treatments have exerted no appreciable influence on the bacterial types. However the application of the green manure has caused considerable disturbance in the equilibrium existing between the various bacterial types. The rod forms have increased from 42 per cent of the total to 62 per cent, largely at the expense of the coccus forms. The spore formers have decreased from 40 per cent to 32 per cent, and the gelatin liquefiers have increased from 78 per cent to 86 per cent.

While these results cover only one small angle of a very large problem, they are indicative of what may be accomplished in the future. Each year it is becoming more apparent that our study of the soil flora must be directed toward a more thorough knowledge of the bacterial types and particularly the interrelationships existing between the various groups.

