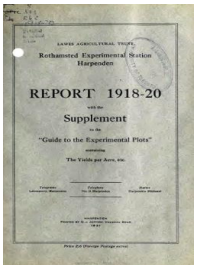
Report 1918-20 With the Supplement to the Guide to the Experimental Plots Containing the Yields per Acre Etc.



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Protozoological Department XIV-xvii

Rothamsted Research

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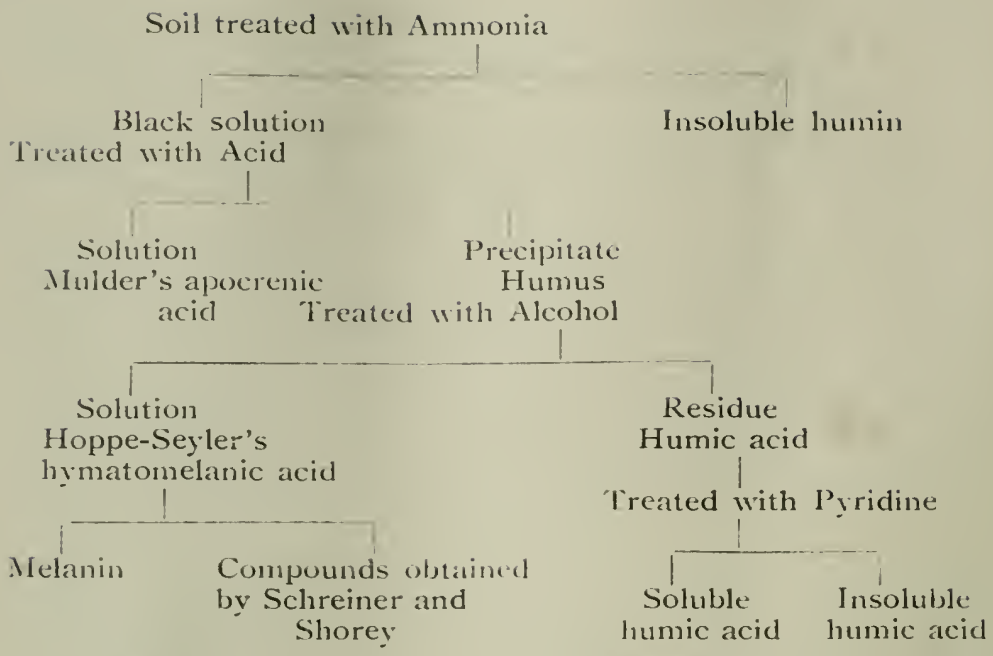

The above procedure has been repeated with rotted straw and with sugar humus, and in both cases similar fractions were obtained. The residue after pyridine extraction of sugar humus was, however, only slowly soluble in ammonia, probably having been converted into humin.

SOIL ORGANISMS.

XIV. L. M. Crump. "*Numbers of Protozoa in certain Rothamsted Soils.*" Journal of Agricultural Science, 1920. Vol. X. pp. 182-198.

The method used was an improvement on that previously adopted in this laboratory, but it did not discriminate between active and encysted forms. Determinations were made at intervals of about seven days of the numbers of total protozoa and bacteria in the soil of Broadbalk Plot 2, which receives 14 tons of farmyard manure in each year, and of Harpenden Field, which is typical of poor arable land. The results are plotted on curves from a study of which the following conclusions are drawn :—

1.—Flagellates, amœbæ and thecamœbæ are usually present in these soils in the trophic condition and in comparatively large numbers, so that there is an extensive population actively in search of food.

2.—The protozoan fauna is practically confined to the top six inches of the soil.

3.—There is a definite inverse relation between the numbers of bacteria and amœbæ.

4.—The amœbæ are uninfluenced by variations in the water content and temperature of the soil and by the rainfall.

5.—The richer the soil is in organic matter the richer it is in protozoa, especially in amœbæ and thecamœbæ.

These conclusions are at variance with those arrived at by the American investigators, but it is believed that the methods employed are better than those used in America.

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XV. D. W. Cutler. *"A Method for Estimating the Number of Active Protozoa in the Soil."* Journal of Agricultural Science, 1920. Vol. X. pp. 135-143.

This method constitutes a great advance on those previously in use, since it discriminates between active and encysted forms; it has, therefore, been adopted in all the succeeding work. The soil is passed through a 3mm. sieve and two samples of 10 grams each are taken. In one the total number of protozoa (active forms plus cysts) is determined as follows: 10 grams of the sieved soil are added to 100 cc. of sterile tap water or physiological salt solution. This gives a 1/10 dilution. From it further dilutions are made as shown below.

No. 1	. .	10 gm. soil	in	100	cc.	H_2O	=1/10	dilution.
2	. .	10 cc.	No. 1 ,,	90	,,		=1/100	,,
3	. .	5 ,,	,, 2 ,,	45	,,		=1/1,000	,,
4	. .	20 ,,	,, 3 ,,	30	,,		=1/2,500	,,
5	. .	20 ,,	,, 4 ,,	20	,,		=1/5,000	,,
6	. .	30 ,,	,, 5 ,,	15	,,		=1/7,500	,,
7	. .	30 ,,	,, 6 ,,	10	,,		=1/10,000	,,
8	. .	20 ,,	,, 7 ,,	30	,,		=1/25,000	,,
9	. .	20 ,,	,, 8 ,,	20	,,		=1/50,000	,,
10	. .	30 ,,	,, 9 ,,	15	,,		=1/75,000	,,
11	. .	30 ,,	,, 10 ,,	10	,,		=1/100,000	,,

Nutrient agar is poured into sterile Petri dishes. When the medium has solidified, the dishes are inoculated in pairs with 1 cc. of each dilution. Incubation at 20° is continued for 28 days, and the plates examined at intervals of 7 days, 14 days, 21 days and 28 days. This long period of incubation is necessary in order to ensure accurate results.

In the other 10 gram sample the cysts only are determined, advantage being taken of the fact that they survive treatment with 2% hydrochloric acid while active forms do not. The soil is therefore treated with sufficient 2% HCl to neutralise the carbonate present and still leave an excess of unchanged 2% acid. The acid is allowed to act overnight. After treatment, the number of protozoa in the sample is ascertained by the dilution method; this gives the number of cysts since the acid has killed all the active forms, leaving most of the cysts unharmed. The number of cysts subtracted from the total number of organisms given by the first count gives the number of active protozoa per gram of the soil sample.

XVI. D. W. Cutler and L. M. Crump. *"Daily Periodicity in the Numbers of Active Soil Flagellates, with a brief note on the Relation of Trophic Amœbæ and Bacterial Numbers."* Annals of Applied Biology, 1920. Vol. VII. pp. 11-24.

Using the preceding method, it was found that the numbers of protozoa varied so rapidly that weekly counts did not fairly represent the changes taking place. A series of daily counts was there-

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fore projected and continued for 28 days—from February 9th to March 8th. During the last 14 days the bacteria also were counted. The following conclusions were drawn:—

1.—There is a daily variation in the number of trophic forms of the three species of flagellates, *Oicomonas sp.* (Martin), *Cercomonas Longicauda* and *Bodo sp.*, in the soil of arable fields.

2.—The numbers of bacteria and trophic amœbæ in the soil are correlated, varying inversely over a period of 14 days.

3.—Temperature and rainfall appear to have no influence on the number of active protozoa in the soil.

(Note.—In view of the importance of these results counts were begun on July 4th, 1920, and have gone on daily ever since: it is proposed to continue these for 365 consecutive days.)

XVII. D. W. Cutler. "*Observations on Soil Protozoa.*" Journal of Agricultural Science, 1919. Vol. IX. pp. 430-444.

It is shown that soil possesses a remarkable power of retaining protozoa. When a suspension of protozoa is shaken with soil all the organisms are withdrawn until the saturation point is reached, after which, for the first time, the supernatant liquid contains protozoa. Some of the results are:—

	Active flagellates and amœbæ millions per c.c. of suspension			
Before shaking with soil	.56	1.64	1.98	2.80
After „ „ „	Nil.	Nil.	0.29	1.04
Number taken up per gram of soil .	All	All	1.69	1.76

Until the soil has absorbed 1.7 millions per gram there is complete retention of the organisms.

One gram of coarse sand is capable of withdrawing approximately 145,000 amœbæ and flagellates from a suspension of any strength. Fine sand withdraws approximately 980,000: soil and partially sterilised soil 1,650,000, ignited soil 1,500,000, and clay 2,450,000 per gram of material in each case.

These figures are constant for given material and organisms, and are independent of the concentration of the suspensions, the time of action, or whether the suspension contains cysts or active forms of the amœbæ and flagellates investigated. Also the action is the same when the experiment is performed with a suspension of living or dead organisms.

Experiments with the ciliate—*Colpoda cucullus*—show that coarse sand retains 27,000; fine sand 185,000; soil and partially sterilised soil 270,000 and clay 450,000 per gram of material.

The importance of this work arises from the fact that some of the previous investigators have examined soil suspensions under the microscope for protozoa, and have drawn certain conclusions from failure to find active forms. The present investigation shows that the method is unreliable and the conclusions, therefore, not

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