

this we then estimated the percentage absorption of infra-red and ultra-violet rays by such a plate.

No. of glass.	Thickness.	Visual.	Infra-red.	Ultra-violet.
	mm.			
256	2·61	50	10·7	26·4
246	0·86	50	18·6	20·4
217	1·55	50	6·61	14·3

Glass 217 would, therefore, appear to be the most efficient in removing rays likely to injure the eye. Its colour is a pale green, very pleasant to use, and the eye quickly becomes accustomed to the slight coloration. Colour matches appear to be but little affected by it.

Soil Protozoa and Soil Bacteria.

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In a paper recently published by Mr. Goodey* it is definitely asserted that ciliates, amœbæ and flagellates cannot function as a factor limiting the numbers of bacteria in soils. It does not appear to me that this conclusion is justified by the experimental data given in this paper, and in view of the importance of the subject it seems desirable to bring together the main facts so far ascertained and to summarise the present position of the problem.

Soil consists of irregular mineral particles of sizes varying from about 1 mm. diameter downwards, together with a smaller proportion of organic substances of varying degrees of complexity, nutrient and other salts, and the oxides of iron, aluminium, and silicon in a form easily soluble in acids or alkalis. The action of the natural processes tends on the whole to effect intermingling of these constituents, at any rate throughout the top 6 inches.

In its physical properties soil behaves like a colloid; it possesses strong powers of absorption, and the phenomena are exactly parallel with those shown by other colloids; it influences the evaporation of water so that the curves become wholly different from those obtained from a water surface or from sand. The evidence all shows that the colloidal constituents are not segregated but are distributed over the surface of the mineral particles. Thus the soil may be looked upon as a mineral framework coated with a

* 'Roy. Soc. Proc.' B, vol. 88, pp. 437-456.

complex mixture of easily soluble oxides of iron, aluminium, and silicon, organic substances, nutrient salts, etc., and behaving physically like a colloid.

About one-third to one-half of the volume of the soil consists of pores into which air diffuses fairly readily, so that the percentage of oxygen is almost the same as in the atmosphere, although that of carbon dioxide is higher, ranging up to 1 per cent. instead of 0.03 per cent. These pores also contain water, the volume of which normally varies from 15 to 30 per cent. of the total volume of the soil. This is mainly distributed in films over the substances coating the mineral particles, and in proportion to those coatings the volume of water is, of course, considerably greater than is here indicated.

The soil is known to be inhabited by numbers of bacteria, eelworms, Vermes and numerous other organisms of higher orders and visible dimensions; all these lead active lives. Recently it has been shown in the Rothamsted Laboratory that a protozoan fauna also exists, some members at least of which are leading a trophic existence. The investigations on the partial sterilisation of soil indicate that the activity of these trophic forms is one of the factors limiting the number of bacteria in the soil and consequently the amount of decomposition they effect. It is this conclusion that is controverted by Mr. Goodey in his recent paper.

The experimental results leading up to this conclusion are as follows* :—

1. Partial sterilisation of soil, *i.e.* heating to a temperature of 60° C. or more, or treatment for a short time with vapours of antiseptics such as toluene, causes first a fall then a rise in bacterial numbers. The rise sets in soon after the antiseptic has been removed and the soil conditions are once more favourable for bacterial development; it goes on till the numbers considerably exceed those present in the original soil.

2. Simultaneously there is a marked increase in the rate of accumulation of ammonia. This sets in as soon as the bacterial numbers begin to rise, and the connection between the two quantities is normally so close as to indicate a causal relationship; the increased ammonia production is, therefore, attributed to the increased numbers of bacteria. There is no disappearance of nitrate; the ammonia is formed from organic nitrogen compounds.

3. The increase in bacterial numbers is the result of improvement in the soil as a medium for bacterial growth and not an improvement in the bacterial flora. Indeed the new flora *per se* is less able to attain high numbers than the old. This is shown by the fact that the old flora when reintroduced into partially sterilised soil attains higher numbers and effects more decomposition than the new flora. Partially sterilised soil plus 0.5 per cent. of untreated

* The details are given in two papers by Russell and Hutchinson in 'Journ. Agric. Sci.' vol. 3, pp. 111-144 (1909), and vol. 5, pp. 152-221 (1913).

soil soon contains higher bacterial numbers per gramme and accumulates ammonia at a faster rate than partially sterilised soil alone.

4. The improvement in the soil brought about by partial sterilisation is permanent, the high bacterial numbers being kept up even for 200 days or more. The improvement, therefore, did not consist in the removal of the products of bacterial activity, because there is much more activity in partially sterilised soil than in untreated soil. Further evidence is afforded by the fact that a second treatment of the soil some months after the first produces little or no effect.

It is evident from (3) and (4) that the factor limiting bacterial numbers in ordinary soils is not bacterial, nor is it any product of bacterial activity, nor does it arise spontaneously in soils.

5. But if some of the untreated soil is introduced into partially sterilised soil, the bacterial numbers, after the initial rise (see (3)), begin to fall. The effect is rather variable, but is usually most marked in moist soils that have been well supplied with organic manures; *e.g.*, in dunged soils, greenhouse soils, sewage farm soils, etc. Thus the limiting factor can be reintroduced from untreated soils.

6. Evidence of the action of the limiting factor in untreated soils is obtained by studying the effect of temperature on bacterial numbers. Untreated soils were maintained at 10°, 20°, 30° C., etc., in a well moistened aerated condition, and periodical counts were made of the numbers of bacteria per gramme. Rise in temperature rarely caused any increase in bacterial numbers; sometimes it had no action, often it caused a fall. But after the soil was partially sterilised the bacterial numbers showed the normal increase with increasing temperatures. Similar results were obtained by varying the amount of moisture but keeping the temperature constant (20° C.). The bacterial numbers in untreated soil behave erratically and tended rather to fall than to rise when the conditions were made more favourable to trophic life; on the other hand, in partially sterilised soil, the bacterial numbers steadily increased with increasing moisture content. Again, when untreated soils are stored in the laboratory or glasshouse under varying conditions of temperature and of moisture content the bacterial numbers fluctuate erratically; when partially sterilised soils are thus stored the fluctuations are regular.

7. When the curves obtained in (6) are examined it becomes evident that the limiting factor in the untreated soils is not the lack of anything* but the presence of something active.

* The soils included fertile loams well supplied with organic matter, calcium carbonate, phosphates, etc.

8. This factor, as already shown, is put out of action by antiseptics and by heating the soil to 60° C., and once out of action it does not reappear. Less drastic methods of treating the soil put it out for a time, but not permanently: *e.g.*, heating to 50°, rapid drying at 35°, treatment with organic vapours less toxic than toluene (*e.g.*, hexane), incomplete treatment with toluene. In all these cases the rise induced in the bacterial numbers per gramme is less in amount than after toluene treatment and is not permanent; the factor sets up again. As a general rule, if the nitrifying organisms are killed the limiting factor is also extinguished; if they are only temporarily suppressed the factor also is only put out for a time.

9. The properties of the limiting factor are:—

(a) It is active and not a lack of something (see (7)).

(b) It is not bacterial (see (3) and (4));

(c) It is extinguished by heat or poisons, and does not reappear if the treatment has sufficed to kill sensitive and non-spore-forming organisms; it may appear, however, if the treatment has not been sufficient to do this.

(d) It can be reintroduced into soils from which it has been permanently extinguished by the addition of a little untreated soil.

(e) It develops more slowly than bacteria, and for some time may show little or no effect, then it causes a marked reduction in the numbers of bacteria, and its final effect is out of all proportion to the amount introduced.

(f) It is favoured by conditions favourable to trophic life in soil, and finally becomes so active that the bacteria become unduly depressed. This is one of the conditions obtaining in glasshouse "sick" soils.*

It is difficult to see what agent other than a living organism can fulfil these conditions. Search was, therefore, made for larger organisms capable of destroying bacteria, and considerable numbers of protozoa were found. The ciliates and amœbæ are killed by partial sterilisation. Whenever they are killed the detrimental factor is found to be put out of action, the bacterial numbers rise and maintain a high level. Whenever the detrimental factor is not put out of action the protozoa are not killed. To these rules we have found no exception. Further, intermediate effects are obtained when a series of organic liquids of varying degrees of toxicity is used in quantities gradually increasing from small ineffective up to completely effective doses. The detrimental factor is not completely suppressed but sets up again after a time, so that the rise in bacterial numbers is not sustained. But the parallelism with ciliates and amœbæ is still preserved: they are completely killed when the detrimental factor is completely put

* This is dealt with fully in 'Journ. Agric. Sci.,' vol. 5, pp. 27-47, 86-111 (1912).

out of action; they are not completely killed, but only suppressed to a greater or less degree, when the detrimental factor is only partly put out of action.*

Now this parallelism between the properties of the detrimental factor and the protozoa is not proof that the protozoa constitute the limiting factor, but it affords sufficient presumptive evidence to justify further examination. The obvious test of adding cultures of protozoa to partially sterilised soil was made, but no depression in bacterial numbers was obtained, instead there was sometimes a rise. But in view of the history of investigations on malaria and other protozoan diseases no great significance was attached to this early failure.

No attempt had been made in any of the above experiments to identify the protozoa or even to ascertain whether any particular form existed in the soil in the trophic state or as cysts. The variety of forms was considerable, and it soon became evident that a definite protozoological survey of the soil was required.

This was accordingly put in hand. In order to give the survey as permanent a value as possible the investigations were not confined to the narrow issue whether soil protozoa do or do not interfere with soil bacteria, but they are put on the broader and safer lines of ascertaining whether a trophic protozoan fauna normally occurs in soil, and, if so, how the protozoa live, and what is their relation to other soil inhabitants.

The first experiments, made by Goodey,† indicated that the protozoa were present only as cysts. Subsequent investigations, however, by Martin and Lewin have established the following conclusions.‡

1. A protozoan fauna in a trophic state normally occurs in soils.
2. The trophic fauna found in the soil differs from that developing when soil is inoculated into hay infusions: the forms which appear to predominate in the soil do not predominate in the hay infusions, and *vice versa* the forms predominating in the hay infusions do not necessarily figure largely in the soil.
3. The trophic fauna is most readily demonstrated, and is therefore presumably most numerous, in moist soils well supplied with organic manures, *e.g.*, in dunged soils, greenhouse soils, sewage soils and especially glasshouse "sick" soils.

It is obvious that the protozoa which live largely on bacteria must function as a factor limiting the numbers of bacteria. The problem is therefore reduced

* Buddin, 'Journ. Agric. Sci.,' vol. 6, pp. 417-451 (1914).

† Goodey, 'Roy. Soc. Proc.,' B, vol. 84, p. 165 (1911).

‡ Martin and Lewin, 'Phil. Trans.,' vol. 205, pp. 77-94 (1914), and 'Journ. Agric. Sci.,' vol. 7, pp. 106-119 (1915).

to finding out how numerous they are and how their activity varies with the varying conditions obtaining in the soil.

Considerable difficulties arise in attempting to enumerate the protozoan fauna, and the line of attack adopted in our laboratory is to study the natural history of the trophic forms in the soil. But the attempt at enumeration has been made recently by Cuningham* in Löhnis's laboratory, with the following results:

4. Tentative minimum estimates made by a dilution method show that the trophic forms are to be numbered at least in thousands per gramme of soil.

(Bacteria commonly occur at the rate of 4 to 10 millions per gramme of soil).

5. A protozoan fauna introduced under suitable precautions into partially sterilised soil effected a considerable reduction in bacterial numbers.

This is as far as the protozoological investigations have gone at present. Cuningham's experiments are being repeated in our laboratory. For the rest, the work is not in a sufficiently advanced state to justify any conclusions as to the part played by the protozoa in the soil, but it has definitely revealed the presence of a trophic fauna and shows that the forms are of considerable interest.†

We can now turn to the criticism urged by Goodey which in his view is sufficiently cogent to upset these conclusions.

Goodey inoculated cultures of various Colpoda (*C. cucullus*, *C. maupasii*, *C. steinii*), a Vorticella (*V. microstoma*), and an unidentified amoeba and a flagellate, into partially sterilised soils free from protozoa, and made periodical counts of the numbers of bacteria. The numbers fell off, but not to any greater extent than in similar soils to which no additions of protozoa were made. He therefore concludes that ciliates, amoebæ, and flagellates cannot be included in the biological factor limiting the number of bacteria in soil.

Two objections can be urged against Goodey's experiments.

1. The organisms inoculated into the soil are in the main those which figure largely in cultures made by adding soil to hay infusions. It has already been shown, however (*v. supra*), that the *culture* fauna is distinct from the *trophic soil* fauna. There is therefore no evidence that the normal soil fauna was put back into the partially sterilised soil: on the contrary it apparently was not. Nor is there evidence, except perhaps in one case, that the added organisms survived at all.

2. The difficulty of securing an adequate control is very great and does not appear to have been overcome. When a soil is partially sterilised either by

* 'Centr. Bakt. Par.,' August 1914, and 'Journ. Agric. Sci.,' vol. 7, pp. 49-74 (1915).

† See for example, the paper by Thornton and Smith, "On Certain Soil Flagellates,"

'Roy. Soc. Proc.,' B, vol. 88, pp. 151-165 (1914).

heat, antiseptics, or prolonged storage, other changes are produced besides the destruction of the limiting factor and the protozoa. Some ammonia is formed and the amount of soluble matter is increased—both evidence of a change in the soil constituents—and within a few days after remoistening great numbers of bacteria and of their decomposition products accumulate.

Thus the two systems are:—

- (1) Untreated soil containing normal numbers of bacteria and protozoa.
- (2) Partially sterilised soil, changed somewhat, and containing abnormal numbers of bacteria and accumulation of their products.

It is obvious that the addition of protozoa to (2) does not make it equal to (1), even if the added protozoan fauna were identical with that in (1) and had an equal chance of growth. But Cuninghame shows it has not, for he finds that the development of protozoa in a medium containing exceptionally large numbers of bacteria is considerably hindered. It is significant also that some 5 per cent. of untreated soil has to be added to partially sterilised soil in order to reintroduce the factor detrimental to bacteria. Nor is the apparently simple case of introducing a protozoan fauna into partially sterilised soil much better. The protozoa are not obtained in pure culture alone; they are added along with hay infusion and bacteria. Thus the two systems are:—

- (1) Partially sterilised soil containing high numbers of bacteria.
- (2) Partially sterilised soil containing high numbers of bacteria + hay infusion + added bacteria + protozoa.

These considerations show that no clear issue is obtained between soil protozoa on the one hand and soil bacteria on the other. Goodey's failure to observe any reduction in numbers in the circumstances cannot, therefore, be taken to justify the conclusion that ciliates, amoebæ, and flagellates do not limit the number of bacteria. Until more is known of the kinds of protozoa occurring in the trophic state in the soil, and of their life-history in the soil, it will not be possible to lay much stress on the negative results of re-infections: on the other hand, Cuninghame's experiments indicate that positive results may be looked for in the near future.
