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## Report 1925-26 With the Supplement to the Guide to the Experimental Plots



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### The Soil Population and Its Behaviour

#### Rothamsted Research

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- XLII. V. SUBRAHMANYAN. "*The Biochemistry of Water-logged Soils, Parts I. and II.*" *Journal of Agricultural Science*, (in the press).

The work described in these papers constitutes the first portion of a systematic investigation into the chemical processes occurring in water-logged soils. This is a subject of great importance in relation to tropical agriculture, in particular, for rice growing. Part I deals with the influence of water-logging on the different forms of nitrogen, on the reaction, on gas production, and on bacterial numbers. The only prominent change in the nitrogen compounds is an increase in the ammonia, which causes a slightly more alkaline reaction. The absence of appreciable carbon-dioxide production, and the lack of any increase in bacterial numbers under aerobic or anærobic conditions, suggests that the ammonia production is due to enzyme action.

Part II describes work confirming this hypothesis. It is shown that ammonia production is not hindered by the presence of an antiseptic, and that the aqueous glycerine extract of toluened soil contains an agent which is able to produce ammonia from simple protein derivatives. An active deaminase preparation, of a protein like nature was isolated. The presence of this deaminase in cultures of soil organisms was demonstrated, and its action on a number of amino-acids was studied. It is concluded that this enzymatic deamination may play an important part in plant nutrition on waterlogged soils.

(f) CHEMICAL ANALYSIS.

- XLIII. C. W. B. ARNOLD. "*Studies on the Carbon and Nitrogen Cycles in the Soil. III. The Determination of Organic Carbon in Soils and Soil Extracts.*" *Journal of Agricultural Science*, (in the press).

A description of the methods of analysis developed for use in the work described in the paper, No. XXXIX.

- XLIV. V. SUBRAHMANYAN. "*An Improved Method for the Determination of Dissolved Oxygen in Water.*" *Journal of Agricultural Science*, (in the press).

This paper deals with methods which have been specially worked out for use in the investigations on waterlogged soils, described in paper No. XLII.

IV. THE SOIL POPULATION & ITS BEHAVIOUR.

(Bacteriological, General Microbiology, Mycological Departments.)

(a) BACTERIA.

- XLV. P. H. H. GRAY. "*A Method of Staining Bacterial Flagella.*" *Journal of Bacteriology*, 1926, Vol. XII., pp. 273-274.

A simplified method that has proved of great value in a procedure, usually attended with much difficulty, essential in bacterial diagnosis.

XLVI. H. G. THORNTON and R. A. FISHER. "On the Existence of Daily Changes in Bacterial Numbers in American Soil." *Soil Science*, 1927, Vol. XXIII., pp. 253-259.

The daily bacterial counts published by Smith and Worden show variations which cannot be explained as being due to sampling errors. On all three media employed by them, significant positive correlations in bacterial numbers between simultaneous samples were obtained.

The similar daily fluctuation occurring in different parts of the plot show most clearly on Thornton's mineral salts medium.

Provided the manipulative technique of Smith and Worden was sufficiently uniform, the results afford evidence of the existence, in very different conditions, of daily fluctuations in bacterial numbers, similar to those observed at Rothamsted.

XLVII. H. G. THORNTON and N. GANGULEE. "The Life-Cycle of the Nodule Organism, *Bacillus radicolica* (Beij), in Soil, and its Relation to the Infection of the Host Plant." *Proceedings of the Royal Society*, 1926, Ser. B., Vol. XCIX., pp. 427-451.

By means of a modification of Winogradsky's staining technique, the changes in morphology of *Bacillus radicolica* in soil were followed. A regular cycle of changes was found, unbanded rods, cocci, and banded rods successively predominating in the soil. Increase in the percentage of cocci was associated with increased bacterial numbers and with the appearance of motile forms.

By modifying the liquid used to suspend the inoculum added to the soil, the time of appearance of cocci in predominance could be altered. In particular, inoculation of the soil with a bacterial suspension in milk containing 0.1 per cent.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$ , hastened the predominance of cocci and increased the percentage to which they attained.

When the centre of a petri dish of soil and sand is inoculated with a suspension of the bacteria, the latter commence, after a lag period, to spread radially at an approximate rate of one inch in 24 hours. The length of this lag period is apparently related to the time taken for cocci to predominate in the soil and is effected by the nature of the inoculating fluid. The bacteria multiply rapidly in the soil into which they have recently spread, so that the nature of the inoculating fluid also exerts an influence on bacterial numbers at a distance from the point of inoculation. Thus inoculation of the soil with a bacterial suspension in milk containing 0.1 per cent.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$ , results in a greater spreading of the bacteria through the soil and in greater multiplication at a distance from the point of inoculation than in the case when soil is inoculated with a suspension in milk alone.

Lucerne plants grown from seed inoculated with a suspension of bacteria in milk containing 0.1 per cent.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$ , showed a considerable increase in nodule numbers and in yield compared with plants from seed inoculated with a suspension in milk alone.

This effect was confined to the deeper portions of the root and therefore increased as the plants became older and roots developed in the deeper soil. This suggests that the additional nodule formation is due to the known effect of the phosphate in increasing the spread of the bacteria.

XLVIII. N. GANGULEE. "*The Effect of Some Soil Conditions on Nodule Formation on Crotalaria juncea. (L).*" *Annals of Applied Biology*, 1926, Vol. XIII., pp. 244-255.

From pot culture experiments, it has been shown that the formation of nodules on the roots of *Crotalaria juncea* is affected significantly by variations in the texture, moisture content, and reaction of the soil, all other conditions being kept uniform. Nodule formation was increased by higher moisture content, by increased coarseness and by reduced hydrogen ion concentration.

XLIX. N. GANGULEE. "*The Organism forming Nodules on Crotalaria juncea (L).*" *Annals of Applied Biology*, 1926, Vol. XIII., pp. 256-259.

The observations recorded in this paper show that the stages which have previously been known to take place in the life cycle of *B. radiculicola* from certain leguminous plants, occur also in the life cycle of the organism isolated from nodule of *Crotalaria juncea*. These changes occur both in solid and in liquid media. The coccoid bodies have been observed both in the non-motile ("Pre-swarmer") and the motile ("Swarmer") stages. These are succeeded by the short-rod stage, which eventually gives rise to the banded (vacuolated) forms. The outset and duration of these stages varies with the composition of the media.

L. N. GANGULEE. "*Studies on the Lucerne Nodule Organism (B. radiculicola) under Laboratory Conditions.*" *Annals of Applied Biology*, 1926, Vol. XII., pp. 360-373.

It is observed that on whatever media the organism is growing, whether in liquid or agar or in soil, the various stages of the life-cycle are found to occur simultaneously, but in varying proportions. The soil conditions, such as aeration, temperature, and the presence of certain salts, are among the factors that determine which of the stages shall be in predominance. The relative efficiency of liquid and solid media in bringing about the predominance of a particular form was also studied.

In the main, the existence of the five stages in the life-cycle of the organism observed by Bewley and Hutchinson is confirmed; but evidence is obtained to show that under very favourable conditions the motile cocci ("swarmer") can emerge directly from the banded rod stage, developing flagella even before their emergence, and therefore missing out the non-motile ("pre-swarmer") stage.

The appearance of coccoid bodies was accelerated in the presence of saccharose, mannite and phosphates (in agar media), and also in soil extract alone. Short rods are elongated cocci, and they tend to persist in media containing the ingredients mentioned above.

(b) PROTOZOA.

- LI. D. W. CUTLER and L. M. CRUMP. "*The Influence of Washing upon the Reproductive Rate of Colpidium colpoda.*" *Biochemical Journal*, 1926, Vol. XIX., pp. 450-453.

The rate of reproduction of *Colpidium colpoda* under various external conditions was described in earlier papers, summarised in the previous report of this station. The present paper gives the results of experiments carried out to test the contention of Prof. T. Brailsford Robertson that a preliminary washing of the organisms before isolation would have modified the results previously obtained.

It was found, however, that this was not the case, no retardation of the reproductive rate being observed.

- LII. D. W. CUTLER and D. V. BAL. "*Influence of Protozoa on the Process of Nitrogen Fixation by Azotobacter chroococcum.*" *Annals of Applied Biology*, 1926, Vol. XIII., pp. 516-534.

Increased nitrogen fixation by *Azotobacter chroococcum* in the presence of protozoa has been shown to occur, as previously described by Nasir (*Annals of Applied Biology*, Vol. X., pp. 122-133).

There is a definite relationship between the efficiency of the strain used, the incubation period, and the concentration of mannitol used.

The feeding action of *Colpidium colpoda*, and *Hartmanella hyalina* on *Azotobacter* has been demonstrated.

The reason for increased nitrogen fixation appears to be due to the efficiency of *Azotobacter* being maintained for a longer period as a result of the feeding action of the protozoa, together with the conservation in the bodies of the protozoa of the nitrogen fixed by the bacteria on which the protozoa have fed.

- LIII. D. W. CUTLER. "*Methods in Soil Protozoology.*" *Abderhalden's Handbuch der Biologischen Arbeitsmethoden*, Urban and Schwartzberg, Berlin.

An account is given in German of the modern technique used for the study of soil protozoa.

- LIV. H. SANDON. "*The Methods and Present State of the Study of Soil Protozoa.*" *Uspiechin Biologitsheskieh Nauk*, 1927.

In this paper a detailed account of the present day technique of soil protozoology is given, together with a brief description of the more recent developments of the subject. It forms one of a series of papers on the modern methods of soil research, edited by Professor Omelianski for the benefit of investigators in Russian-speaking countries.

(c) FUNGI.

- LV. F. CHODAT. "*Recherches Expérimentales sur la Mutation chez les Champignons.*" Bulletin de la Société Botanique de Genève, 1926, Serie 2, Vol. XVIII., pp. 41-144.

Out of over 80 species of fungi grown in culture in order to observe the relative occurrence of genetic instability, two species, *Aspergillus ochraceus* and *Phoma alternariacearum* were selected for detailed study.

*Aspergillus ochraceus*.—From single-spore cultures, two strains of this fungus were obtained, one the original yellow form and the other, originating as a sector, a dwarfed form, producing a red colour in the medium. These types proved to be constant for many generations over a long period. Morphologically, the two forms were distinct in several characters. The physiological properties of the fungi were examined in detail and correlations were found between the factors—amount of nitrogen and carbon in the medium, the pH of the latter and the production of the red colouring matter. The growth of the parent form was greater than that of the variant on media poor in nitrogen, the latter requiring more nitrogen to produce less growth. This difference was correlated with the fertility of the yellow strain and the partial sterility of the red strain.

*Phoma alternariacearum*.—From single spore cultures, five distinct forms of this species were ultimately obtained, which remained constant for many generations. A study was made of the morphological, physiological and biometric differences between these forms and general relations found to hold between biometric and cultural data were traced to physiological causation.

The interpretation of the variations observed is discussed in relation to previous work on fungi.

- LVI. WM. B. BRIERLEY. "*Variation in Fungi and Bacteria.*" Transactions of the International Congress of Botany, Ithaca, U.S.A., 1926. In the Press.

An introductory review of the sources of information regarding the present status of the genetics of micro-organisms is followed by a critique of genetic phenomena, concepts and terminology in groups of organisms other than bacteria and fungi. These latter groups are considered in relation to the various methods which may be adopted of classifying genetic data. An outline schedule is presented in which a considerable number of data on variation in fungi and bacteria are arranged according to the time and mode of origin of the variants, their constancy and relation to environmental conditions and their relation to prior and succeeding generations.

- LVII. R. D. REGE. "*Biochemical Decomposition of Cellulosic Materials with Particular Reference to the Action of Fungi.*" Annals of Applied Biology, 1927, Vol. XIV., pp. 1-44.

Two factors appear to control the decomposition of ripe cellulosic material in presence of available nitrogen. One, the

food or energy factor, is usually represented by pentosans; the other, or inhibitory factor, is always lignin. The predominance of the former over the latter determines the ease of decomposition. The results now obtained confirm the view put forward in 1924 that any cellulosic material containing 30 per cent. of pentosan and a relatively small amount of woody fibre can be converted easily into an organic nitrogenous manure. The prediction of the "decomposibility" of a material is thus possible.

Attempts to increase the ratio of energy to inhibitory factor in resistant materials by the addition of carbohydrates proved unsuccessful. It was concluded that since micro-organisms obtained their food materials outside the tissues, they did not attack the tissues until the more easily available food-stuffs were exhausted. Thus the decomposition of the material was actually less than was possible under natural conditions.

Mannose and galactose do not appear to form suitable food for the micro-organisms concerned in these processes, and it is concluded that the pentosan part of the hemi-celluloses is most important as microbial food. Incidentally, it is shown that Kröber and Tollens' method for determining pentosans is untrustworthy and a modification is suggested.

A study of the relative importance of bacteria and fungi proves that, under the conditions of these experiments, most of the work is done by fungi. The high temperature (70° C.) often reached by manure heaps is above the range of such organisms as *Spirochæta cytophaga*, but one of the fungi now isolated flourishes at 50° C. and upwards.

(d) ALGÆ.

LVIII. B. MURIEL BRISTOL ROACH. "On the Relation of Certain Soil Algæ to some Soluble Carbon Compounds." *Annals of Botany*, 1926, Vol. XI., pp. 149-201.

A method is described for obtaining pure cultures of algæ from the soil.

In pure cultures of soil algæ on solid media, the great majority of species show greatly increased growth in the presence of a number of different soluble organic compounds, each species having its own order of selection of the compounds that have been tested; a few species do not behave in this way, and are possibly completely autotrophic in nutrition.

Pure cultures of several soil species in liquid media containing glucose showed that the best estimate of the growth of a unicellular alga may be obtained by making daily measurements of the average size of the cells and of the number of cells per unit volume of liquid, and by calculating from these data the bulk of algal protoplasm present. The logarithmic values of the bulk when plotted against time lie upon a straight line within the limits of experimental error for a limited period of growth; the slope of this line (i.e., the tangent of the angle which it makes with the horizontal axis) may be taken as a measure of the rate of growth of the organism in the given medium.

The growth of the alga *Scenedesmus costulatus*, Chod., var. *chlorelloides*, nov. var., has been studied quantitatively in liquid

media containing mineral salts and 1 per cent. of certain soluble organic compounds. In the glucose medium, the degree of variance of the observed values (logarithmic) from the calculated straight line of nearest fit is shown to be greatly reduced by rigorous control of light and of temperature, and by continuous aeration of the medium. In this medium the organism is able to grow in the dark, retaining its green colour. There is some reason to believe that the rate of growth in the dark may be approximately equal to the difference between its activities in the light in the same medium and in that with mineral salts alone. In certain media containing substances less favourable to the growth of the organism, the degree of deviation of the observed values from the straight line is greater than in the glucose medium. With maltose there appears to be an initial "lag" period preceding the straight-line period of growth. In mannite there are conspicuous fluctuations in the growth rate due to death of the young cells. Xylose is completely toxic to the organism under the conditions observed.

The relative average rates of growth in the different media may be expressed quantitatively, as follows: Glucose in the light 100 per cent., maltose 100 per cent. preceded by a "lag" period, galactose 94 per cent., sucrose 84 per cent., fructose 73 per cent., mineral salts alone 60 per cent., glycerine 43 per cent., glucose in darkness 40 per cent., mannite 13 per cent., xylose 0 per cent.

In those media that are completely favourable to its growth, the increase in bulk of *Scenedesmus costulatus*, Chod., var. *chlorelloides*, follows the same laws as a simple exponential curve, for a limited period of time.

LIX. B. MURIEL BRISTOL ROACH. "Methods for Use in Studying the Algæ of the Soil." in Abderhalden—Handbuch der biologischen Arbeitsmethoden, 1926.

Details are given of a cultural method for estimating roughly the numbers of algæ (Chlorophyceæ and Diatoms only) in the soil. Methods are also described for the isolation and cultivation of soil algæ in (a) impure and (b) pure cultures, and suitable media are recommended for use. An account is given of a special method for estimating quantitatively the effect of any condition or chemical compound on the rate of growth of a unicellular soil alga, the rate of growth being regarded as an index of the metabolism of the organism.

Methods are also described for studying the biochemical activities of pure cultures of algæ under the following headings:—(1) Decomposition of protein (gelatine); (2) Fixation of nitrogen; (3) Transformation of insoluble mineral substances into soluble forms.

## V. THE PLANT IN DISEASE; CONTROL OF DISEASE.

(Entomological, Insecticides and Fungicides, Mycological Departments.)

### (a) INSECT PESTS AND THEIR CONTROL.

LX. W. M. DAVIES. "On the Tracheal System of *Collembola* with Special Reference to the Species *Sminthurus viridis*." Quarterly Journal of Microscopical Science, 1927, Vol. CXXI., pp. 15-30.