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SOIL FERTILITY.

Micro-Organisms Explained.

By Professor J. A. Prescott before the Field Naturalists' Society.

In speaking to field naturalists about soils, I feel sure I am speaking on a subject in which you take a lively interest. Whether your interests are in geology, botany, or zoology, sooner or later some aspect of soil science is bound to crop up. To the geologist, soil is the final product in the break up of the original rocks of the country. For many years soil was considered in fact to be simply a mass of rock material divided up into various grades of fineness. This conception is a useful one as far as it goes, but there are additional conceptions which of recent years have become more and more important. The fineness of this breakdown in itself brings in a new set of properties, which are of great interest to the chemist and physicist, while at the same time the soil is no longer a dead mass of broken stone, but has become the dwelling place of countless millions of organisms whose activities are of great importance to the plants which grow on that soil. When dead or waste animal or vegetable material is left about, you are all aware that in time it will eventually disappear all the more quickly if it is buried in the ground. In this way the stubble of the crops, the leaves and dead branches of the forest, the animals that perish, all disappear. If this were not the case, the earth would soon be encumbered with waste material, which could only be got rid of by a periodical burning off. The gradual decay of material is in fact recognised by the chemists as simply a slow burning away. For many years this decay was in fact treated as purely chemical, and it was not until Pasteur's brilliant researches into micro-organisms that it became evident that all these breakings down and slow combustions were in fact the result of their activities.

Important Constituents of Plants.

In this breaking up two elements are mainly involved—nitrogen and carbon. Both are important constituents of plants, and nitrogen in particular is a valuable plant food, which is supplied by the soil, while carbon as you know reaches the plants from the carbon dioxide of the atmosphere. The carbohydrates, which consist of sugars, starch, and cellulose—which is the raw material of cotton, linen, or wood—have their own ways of decomposition, but eventually appear again as carbon dioxide to be used over again by new generations of plants. In the soil there is also formed an intermediate product—the black, peaty-like material called humus, which rejoices the heart of every good gardener. This formation of humus from fibre and wood-like material is as yet imperfectly understood, but it is the work of bacteria and fungi. The compounds of nitrogen—proteins they are called—are typified by the white of egg (albumen), gelatine, meat, and the gulten of Wheat. Their break up is an exceedingly interesting one, and the nitrogen of their complicated structures eventually turns up as ammonia or as nitrate of which form the plant can again make use. As the irreverent student once said:—"Let us eat, drink, and be merry, for to-morrow we turn into water, carbon dioxide, and ammonia." Some of these changes or fermentations have been turned to advantage for many centuries as in the manufacture of wine and beer, the deliberate souring of milk and cream in the manufacture of the butter and the drinks of eastern Europe, the ripening of cheese, the manufacture of nitre, and finally the disposal of sewage.

Simplest Form of Plant Life.

Bacteria are the simplest from of plant life. In shape they are round (coccus), rod like (bacillus), screw like (spirillum), or curved (vibrio). In dimensions they are most minute, being little more than a thousandth of a millimeter in diameter, that is one 25,000th of an inch. To see them at all well, it is usually necessary to stain them with aniline dyes, and to use magnifications of a thousand diameters. Although so small, their numbers run into millions per gramme of soil, and it is this numerical strength and the rapidity with which they multiply that enables them to produce such noticeable chemical changes. In order to overcome periods of adverse conditions, many bacteria produce spores or resting cells, which are much more resistant to dryness or high temperature than the parent cell. Bacteria are readily killed off at the temperature of boiling water, or by chloroform and certain antiseptics. The spores, however, are much more resistant, so that to completely sterilize a soil or any medium such as milk, it is necessary either to heat to boiling on several successive days, or to use a much higher temperature or steam under pressure. When milk is pasteurised, it is not completely sterilised, but certain disease organisms which do not produce spores are thereby killed, and so the milk is rendered safe for the time being. When a soil contains so many millions of bacteria of so many different kinds, it would, of course, be difficult to separate them out and examine them individually. When the bacteriologist wishes to do this, he either makes conditions favourable for the particular organism in

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which he is interested, or he dilutes the soil with a large volume of sterile water, and takes a minute portion of this and spreads it out over the surface of a jelly prepared either from gelatine or from agar (provided from seaweed). The fact that the soil contains bacteria can readily be shown by placing a small quantity of soil into sterile milk.

Nitrification.

After it had been shown that the fermentation of sugar to alcohol was the result of the activities of the yeast plant, and the importance of bacteria in controlling certain diseases and fermentations had been demonstrated, Pasteur in 1862 suggested that nitrification would probably turn out to be of a similar character. The fact that certain soils in India and China contained nitre had long been known, and certain districts in Northern India were at one time providers of commercial quantities of saltpetre. In France, Germany, and Sweden it had been found and developed into quite an industry, that, when waste products were composited with the soil and kept under certain conditions of heat and moisture, eventually nitre or potassium nitrate could be washed out of the soil. The French, in fact, developed this into a fine art, and in 1777 published a complete set of instructions concerning the maintenance and control of nitre beds. The process was described by Georgius Agricolo (1494-1555) in his book "De Re Metallica, and it was very important, as these nitre beds were the only known source of saltpetre used in the gunpowder of the wars of the 17th and 18th centuries. In 1877, Schloesing and Muntz took up this problem of nitrification, and showed that it was bound up with the purification of sewage. They prepared a column of sand, through which sewage was allowed to filter. At first there was slight change, but after 20 days change proceeded more rapidly until all the waste nitrogen that went in with the sewage at the top came out at the bottom as nitrate. If the change was purely chemical, why should there have been a delay? This suggested that it was a biological action. They then treated the top of the column with chloroform vapour, and they found that nitrification ceased when the chloroform vapour was removed. There was still no nitrification, but after seeding the top of the sand with a little fresh soil, the process was restored to its former vigour.

Value of Discovery.

Robert Warington, who was working on soil nitrates at Rothamsted at this time, saw at once the value of this discovery from the agricultural point of view, and he succeeded in carrying the work forward with soils. He showed that nitrification took place in two stages—first, ammonia was transformed into nitrite, and then nitrite into nitrate. The organism that was able to accomplish the first stage was unable to complete the next stage, and vice versa. He was unable to isolate the organisms, however, although he and others tried for 10 years. The culture solutions were diluted and spread out on to gelatine plates, but although many organisms developed, not one of these was able to do the work of nitrification. Winogradsky, a young Russian working at the Pasteur Institute, about this time (1890-1892), was finally able to solve this problem. He substituted for the gelatine a jelly consisting entirely of silica, the substance of sand, quartz, and opal, and was able at once to separate out the nitrifying organisms. They were quite unable to live, work, and grow on the rich organic medium that had been tried up to that time. The two organisms are called nitrosomonas and nitrobacter. When the nitrate stage is reached in the soil, the nitrogen has been converted into simplest, useful form and is available as plant food. The waste nitrogenous material is converted first into ammonia by the action of fungi and of many varied species of bacteria, this ammonia is converted at once into nitrite and the nitrite into nitrate. This third process is by far the most rapid, so that it is very difficult to find either ammonia or nitrite in an ordinary soil, only the final stage—the nitrate stage being present. Apart from suitable moisture supply, a summer-like temperature, and the presence of suitable nitrifying raw material, a fourth requisite is the presence of lime in the soil or culture solutions, otherwise the activity soon slows down owing to the production of acid. Of course the other ordinary plant nutrients like phosphates and potassim salts must also be present.

Fixation of Nitrogen.

It has been known for many centuries than when certain leguminous crops such as beans, peas, lupines, and clovers are grown, the soil is usually enriched rather than impoverished as is the case of continuous cropping with cereal crops. The value of clovers in this respect was known to the Romans and Theophrastus, the Greek writer of "Enquiry into Plans" states:—"Of the leguminous plants, the bean best invigorates the ground . . . because the plant is of loose growth and rots easily, wherefore the people of Macedonia and Thessaly turn over the ground when it is in flower." (They dig it into the ground to enrich the soil.) There is little doubt that the ancient Egyptians knew of the value of these crops already. We had no records. The Egyptians were better artists than philosophers. During the early half of the nineteenth century when the first field experiment, started by Boussingault in Alsace, and by Lawes and Gilbert in England, enabled us to produce a chemical balance sheet of the production of the land—it was found that these leguminous crops had some exter-

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nal source of nitrogen. They always produced more nitrogenous material than corresponded to the nitrogen supplied in the manure, and yet left the ground all the richer in this element. The laboratory experiments of Lawes and Gilbert afforded no light on the subject—bacteriology had not yet begun—and they took such precautions to burn the soil and so keep it free from ammonia and organic matter that their clover crops behave just like the wheat crops and refused to grow unless manured in the same way. If the roots of any of these leguminous plants are examined, it will be seen that they are covered by nodules. Lachman in 1858 showed that these nodules contained bacteria, and this had been confirmed by succeeding botanists, but it was not until 1888 that Mellriegel and Wilfarth were able to show the significance of these observations. They were able to show as a result of their experiments with peas:—(1) The peas took their nitrogen from the air; and (2) the process of the nitrogen assimilation was associated with the presence of nodules on the plants and the activities of the bacteria in these nodules.

Nitrogen from the Air.

Later on, two French chemists, Schloesing (fils) and Laurent, were able to show by analysing the air and the plants that this nitrogen fixed by the plants actually came from the air. This process is a typical case of symbiosis—the bacteria take their nitrogen from the air, but their main food supply, such as sugars, from the plants. In exchange the plants get their supply of nitrogen. The nodule organism was first isolated by Beijerinck and called "Bacterium (or bacillus) radicicola." The study of this organism has been taken up at Rothamsted, and it has been shown to follow a life cycle, the stages of which can be speeded up or retarted by external conditions.. Hutchinson and Bewley have been able to give the most complete account of this cycle—the bacteroids found in plants break up into minute cocci. In the presence of sugars and phosphates, these minute sphere begin to form swarmers which have recently been shown to have the power of moving through the soil. These swarmers attack the roots of the leguminous plants and produce the nodules. There are a number of strains of the nodule organisms which are associated with different types of leguminous plants. The organisms form the nodule of the garden pea, are able to produce nodules on broad beans and sweet peas also, but not on lucerne and clover. The bacteria from the nodules of soy beans are able to infect only soy beans, and quite unable to infect other legumes.

Importance of Leguminous Crops.

The leguminous crops are thus of enormous importance to agriculture. They are able to look after their own nitrogen supply, and to enrich the soil at the same time. Nitrogen is the most expensive of all fertilizers, and by the cultivation of leguminous crops in the farm rotation, the cultivator is able to maintain something like a permanent level of soil fertility. When a leguminous crop is introduced into a new country, it is frequently found that the plant grows feebly. It is frequently found that the correct nodule organism is not present in the soil, and matters can be remedied by inoculating the soil with cultures of the correct strain of organism. This inoculation can also be carried out by the use of soil from an old established field of the crop to be grown, and this method is frequently the most satisfactory. Sometimes greater nodule development may be stimulated by improving the soil conditions. Phosphates, in particular, are known to be favourable, and the wonderful effect that phosphates have in the encouragement of leguminous crops in pastures may be due in part indirectly to this stimulating of nodule formation, so enabling the plants to secure more nitrogen, and thus get ahead in the competition with the grasses which prevailed before topdressing was resorted to. Farmyard manure probably, also acts as a stimulus to the nodule organism. Another series of independent organisms capable of fixing nitrogen has also been discovered. They include two types—Clostridium Pasteurianum, which was also discovered by Winogradsky, and which only works in the absence of oxygen, and another type—Azotobacter, which works in the presence of oxygen, and was discovered by Beijerinck. Azotobacter is a very important contributor to soil fertility, although we have very few figures to show exactly how much it does contribute. It requires a source of energy to enable it to live, such as is supplied by starch, sugar, or decaying plant fibres, and it is probably the main source of the high fertility of virgin soils which have never been cropped.

Losses of Nitrogen.

The activities of the soil bacteria are not, however, all of gain to the farmer. Under certain conditions nitrogen already in the soil may be lost in the course of transformation. When soil is waterlogged, for instance, many organisms, feeling the need for more air, so to speak, readily decompose nitrates and cause the nitrogen to be dissipated. Other fermentation taking place under the best of conditions causes a notable loss of nitrogen, which we are not yet in a position to understand, although a considerable amount of work has been done on the subject already. These losses are serious when land is continually cropped without due consideration to the maintenance of a permanent level of fertility.

Partial Sterilization.

In 1907 Sir John Russell (Director of Rothamsted) was working on the chemi-

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cal changes in the soil. He was following the changes in the atmosphere surrounding to the soil particles in order to trace the needs of the bacterial population for oxygen. By some accident, the laboratory assistant, who was to sterilize the soil, forgot to raise the temperature above that of boiling water, so that the soil was only partially sterilized. If the soil had been completely sterilized, all chemical activity should have ceased, but he found that the activity was considerably augmented, so that more oxygen was used up than in the case of original soil. As a result of this chance observation, investigations were carried out at Rothamsted, on this effect of partial sterilization which have developed into an extensive network of work. It was soon found that the effect of partial sterilization was:—1. Bacterial numbers first fell off, but almost immediately rose to levels never before reached in untreated soils. 2. Nitrates were no longer produced, but valuable quantities of ammonia were produced instead, far in excess of the available plant food in the untreated soil. 3. Similar effects could be produced by treating the soil with volatile antiseptics like toluene, carbon bisulphide, or fomalin. 4. Crops grow very much better in partially sterilized than in untreated soils. The result of partial sterilization is evidently to simplify the soil population; nitrofying organisms are killed off, but they can soon reinfect the soil again. It was eventually suggested that the protozoa were the limiting factor to bacteriological activity, and that partially sterilizing the soil killed them off without affecting the bulk of the bacterial population. Whenever the protozoa were killed it was found that the partial sterilization was effective. Much work has been done since that time on the subject of soil protozoa, and they have been found in soils from Spitzbergen to South Georgia, and from the deserts of Egypt. Some types of soil protozoa are remarkable for their uniformly wide distribution. At Rothamsted their daily fluctuations in numbers have been observed for a whole year, and a number of remarkable facts have been established, such as their daily periodicity, and the fact that when the numbers of certain protozoa are high, the numbers of bacteria are low. The soil population, in fact, changes not only from day to day, but from hour to hour. The partial sterilization of the soil has now become an established practice in English glasshouse cultivation of tomatoes and cucumbers. Soils that were considered to be tomato sick, and at one time thrown away, although actually richer than farmyard manure, are now partially sterilized and used over again.

Bacteria in the Field.

The passing of the seasons, the cultivation of crops, the preparation of fallows, all have their effects on the bacterial numbers in the soil. Usually there is an increase in bacterial numbers in the spring and again in the autumn. At Urrbrae, just before the break of weather, there were about two millions of bacteria per gramme of soil, but within a few days of the first rains they rose to 15 millions, dropping later to about eight millions. In the spring, while soil is still moist, but when the temperature begins to rise, the numbers usually increase again. An interesting way to follow these changes is to analyse the soil for nitrates, the actual quantity found being the balance between what the bacteria are produced, what the crops take away, and what is washed down by the rain. When the soil is fallowed, the bacteria are kept busy producing nitrates the whole time; the frequent cultivation aerates the soil and conserves the moisture, so that before the seed is sown large amounts of nitrate have frequently been stored up for the benefit of the crop. Another type of fallow is illustrated by the Egyptian "sharaqi" or thirsty fallow. The soil is so dry that it is impossible to cultivate it. It also becomes very hot, the surface temperature reaching to between 60 and 70 deg. centigrade. This kind of fallow is beneficial in quite another way; there are no changes going on, and hence no wasting of food material. It also acts as a partial sterilization, and when the flood waters are allowed to reach the land, the bacterial numbers rise very rapidly.

Farmyard Manure.

The fermentations that take place in the manure heap, causing it to break down into a brown mass, are also the work of bacteria. The full story is too complicated for us to follow in the limited time at our disposal, but the workers at Rothamsted have been able to reproduce this fermentation artificially by treating straw with a nitrogenous chemical like sulphate of ammonia or calcium cyanamide together with a little phosphate and lime, and to obtain a brown material which behaves in many ways like the old-fashioned manure. This is very important to people like market gardeners, who have been accustomed to use large quantities, for they are having difficulties now that the horse is being replaced on the farm by the tractor and motor car.