IV.—*On Nitrification.*

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I HAVE the pleasure of submitting to the Society a report of some experiments on nitrification recently carried out in the Rothamsted Laboratory.

The formation of nitrates in soil, under favourable conditions as to moisture, temperature, and aëration, is a reaction well known and frequently studied. It is a process of great technical importance, being the only natural source of saltpetre. It is also of the highest interest to the scientific agriculturalist, nitrates being invaluable as plant-food, and their loss by drainage one of the most serious difficulties with which a farmer has to contend.

The bodies yielding nitric acid in the soil are, firstly, the various nitrogenous organic substances which arise from the decay of vegetable or animal matter; and secondly, ammonium salts, either produced in small quantity during the decay of organic matter, or carried to the soil by rain, or, in some cases, applied intentionally as manure. A further source of the nitrates contained in soil is to be found, according to some writers, in the free nitrogen of the atmosphere; but of any supply from this source, other than the ready-formed nitrates contained in rain, there is at present no substantial proof.

The production of nitric acid, both from ammonia, and from the organic matter of arable soil, is well illustrated by the field experiments at Rothamsted. The winter drainage-water from the various plots in the experimental wheatfield, is found to contain nitrates nearly in proportion to the quantity of ammonium-salts applied the preceding autumn (*Jour. Roy. Agri. Soc.*, 1873, 334, 337).* While the drainage-water, rich in nitrates, which is obtained at the drain-gauges from soil which has been unmanured for the last eight years, clearly derives the greater part of its nitrogen from the organic matter of the soil.

Many investigations have been made as to the mode in which nitrification takes place. A mixture of nitrogen and oxygen is known to yield nitrous acid when subjected to repeated electrical discharge, but

* For further details see *Jour. Chem. Soc.*, 1871, 286; also *Sixth Report of the Rivers' Pollution Commission*, 58—68; and a lecture by Dr. Gilbert, at the Science Conference at South Kensington, "On Some Points in connection with Vegetation," page 39.

ozone has been shown to be incapable of oxidising gaseous nitrogen. Ammonia may be converted into nitrous and nitric acid either by electric discharges in the presence of oxygen, or by the action of ozone (L. Carius, *Ann. Chem. Pharm.*, clxxiv, 31), but not by ordinary oxygen. Ammonia may also be oxidised into nitric acid, according to some observers (Pesci, Thenard, Knop), by prolonged contact with ferric oxide at ordinary temperatures. With regard to the production of nitric acid from nitrogenous organic bodies, very little seems to be known. It is generally assumed that when such bodies decay in a porous medium, offering a sufficiently large surface for oxidation, nitrates must necessarily be formed, especially if a salifiable base be present; but this view, as far as I am aware, has never been confirmed by exact experiments. The action of ordinary oxygen upon organic matter is supplemented, in the theory of some writers, by the action of ferric oxide and of ozone.

In February of the present year Schloësing and Müntz laid before the French Academy (*Compt. rend.*, lxxxiv, 301) the results of an experiment, proving, in their opinion, that nitrification was due to the action of an organised ferment; that, in fact, it was probably a function of some low form of vegetable life. This theory of nitrification they further state was regarded as probable by Pasteur as far back as 1862. Pasteur, in view of the active oxidation induced by mycodermis in various kinds of organic matter, expressed the opinion that nitrification required to be studied over again from this point of view. As the experiment of Schloësing and Müntz formed the starting point of the trials at Rothamsted, it will be necessary to give a brief account of their research.

The primary object of these experimenters was to ascertain if the presence of humic matter was essential to the purification of sewage by soil. To this end they took a glass tube one metre in length, and filled it with a mixture of ignited quartz sand and powdered limestone; 5 kilos. of sand, and 100 grams of lime-stone were employed. The column of purely mineral soil thus prepared was supplied with sewage at such a rate that the sewage took eight days to pass through the soil.

During the first 20 days the sewage passed through unaltered; but after that time nitric acid began to appear in the filtrate, and rapidly increased till the filtered sewage contained no longer any ammonia, but nitrates only. The authors ask—If it was a simple case of oxidation, why did 20 days elapse before the commencement of the reaction? If, on the other hand, the seeding and growth of germs was necessary before the soil could become effective, the reason of the delay in nitrification is at once apparent.

After passing sewage four months with complete oxidation of its

ammonia, the authors placed a small vessel of chloroform at the top of the column, and allowed the vapour of the chloroform to pour continuously upon the soil. Müntz had shown in an earlier investigation (*Compt. rend.*, lxxx, 1250), that chloroform effectually suspends the action of organised ferments, while it has little or no action upon soluble ferments. In 10 days after the introduction of chloroform vapour all nitrates had disappeared in the exit water, and the sewage passed through the soil quite unchanged; nitrification had thus plainly been effected by some agent, the activity of which was destroyed by the presence of chloroform.

After supplying chloroform for 15 days, the vessel containing it was withdrawn. Nitrification did not resume with the removal of the chloroform. After about seven weeks, during which the sewage passed unchanged, the attempt was made to seed the column of sand afresh with the organisms necessary for nitrification. For this purpose 10 grams of a vegetable soil, known to nitrify with ease, were washed with water, and the turbid washings poured upon the column of sand. Eight days after this application nitrates again appeared in the exit water. As the sewage took eight days to traverse the column of sand, this was the shortest time in which nitrification could become apparent.

Such is the investigation of Schlöesing and Müntz. They conclude that in the instance before them, and in other cases of rapid nitrification, oxidation is produced through the action of a living organism; but they are careful not to assert that this is the only mode in which nitrification can take place.

The importance of this new theory of nitrification is clearly very great. As the amount of nitrates occurring in soils of known history is at present being investigated at Rothamsted, it was thought advisable to submit the new theory without delay to the test of further experiment. The conclusion of Schlöesing and Müntz is clearly based on two distinct lines of proof. Firstly, the action of antiseptic vapours in preventing nitrification. Secondly, the possibility of inducing nitrification by seeding with a substance already nitrifying. We will describe, in the first place, the experiments made under the former head of inquiry.

A soil was taken from the surface of a kitchen garden, the collection being made after heavy rain, when there was, consequently, but little nitrate present. The soil was air-dried, crushed, and sifted, and the fine soil employed for the experiments. Two series of experiments were made with this soil, but as the general method was in each case the same, one description of the plan followed will suffice.

Four glass tubes were taken, in each of which 100 grams of soil were placed. In the first experiment these tubes were of unequal

length; in the second trial the tubes were all 12 inches in height. In the first experiment the tubes were filled with the dry soil, and 10 c.c. of water gradually added afterwards; the result was that only the upper half of the column of soil became visibly wet. In the second experiment the soil was uniformly mixed with 20 c.c. of water before its introduction to the tubes, the whole of the column was thus in a moist condition. The lower end of each of the four tubes was connected with a small water-pump, which served as an aspirator, while the upper end was connected with a U-tube containing pumice drenched with very dilute sulphuric acid. This U-tube ensured the supply of moist air to the soil, and cut off any ammoniacal vapours that might be present in the laboratory. In the case of three out of the four soil tubes a 10 oz. bottle, containing a few pieces of sponge, and provided with a cork and two tubes, was interposed between the soil tube and that containing pumice and sulphuric acid. The sponge in one of these bottles was moistened with a small quantity of liquid carbolic acid. The sponge of a second bottle received bisulphide of carbon. The third bottle received chloroform. The carbolic acid being little volatile, was not renewed during the course of an experiment. Of the bisulphide of carbon and chloroform 5 c.c. were used on the first day of the experiment, and on every seventh day a fresh quantity of 2 c.c. was placed on the sponge. As these liquids are very volatile, it is evident that during a great part of both experiments the amount of bisulphide of carbon and of chloroform carried to the soil must have been extremely small. The result of the whole arrangement was that through one soil tube moist air free from ammonia could be aspirated; and that in the case of the remaining three tubes the moist air reached the soil more or less charged with antiseptic vapours. Aspiration was carried on for 1 to $1\frac{1}{2}$ hour, through each tube, nearly every day.

The first series of experiments commenced on June 29th, and concluded on August 7th, lasting in all 39 days. The second series commenced on September 24th, and lasted till November 9th, or 46 days.

At the conclusion of both experiments the soil in each tube was extracted with hot water, the chlorine removed with sulphate of silver, and the nitric and nitrous acid present finally measured as nitric oxide gas after the method of Crum and Frankland.* A considerable amount of froth was in most cases produced in the shaking tube, and in some instances the whole of the gas could not be removed from the shaking tube to the eudiometer; the determinations which are too

* The above is but an outline of the process used; the exact mode of treatment found most suitable in determinations of nitrates in soil will probably be described on a future occasion.

low from this cause are marked in the following table with an asterisk. The determinations belonging to the second series of experiments were, as the result of more experience, more accurately done than those in the first series. A summary of the results will be found in the following table:—

Nitrogen as Nitrates and Nitrites per Million of air-dried Soil.

History of Soil.	First Experiment.	Second Experiment.
Original soil.....	6·12*	8·91
Air passed	46·87	50·86
„ with carbolic acid	17·20	40·77
„ „ carbon bisulphide ..	6·70*	9·75
„ „ chloroform	9·48	7·86*

It is evident at once on a review of these figures, that a very large amount of nitrification was determined in each case by aspirating ordinary air through the garden soil, the amount being greatest in the second experiment, in which the soil contained more water, and was equally moistened throughout.

The effect of carbolic acid vapour in preventing nitrification is in both cases imperfect, though much less so in the first experiment than in the second. The fact admits, I believe, of explanation. Carbolic acid enters but slowly into vapour at ordinary temperatures, the vapour is also soluble in water. In the second series of experiments the temperature of the laboratory was lower, the tube containing the soil was longer, and the soil uniformly wet. A smaller amount of carbolic vapour would thus enter the soil than in the first experiment, and the quantity so entering would be retained in the upper portion of the moist soil, while nitrification went on unhindered below. In fact, when the tube was taken down at the end of the experiment, it was found that the cotton wool placed at the lower end of the tube was covered with mould, and no smell of carbolic acid could be perceived; while the wool at the upper end of the tube was quite clean, and the odour of carbolic acid manifest. In the first experiment, as already mentioned, only the upper half of the column of soil was really wet; here nitrification was checked by the carbolic acid, while below nitrification could proceed but slowly from lack of moisture. We learn from this experiment, that carbolic vapour cannot be successfully employed as an antiseptic when any penetration of a moist porous body is demanded, whereas bisulphide of carbon fulfils such requirements excellently. In the case of the soils treated with chloroform and bisulphide of carbon, the cotton wool was found perfectly clean at both ends of the column, and the odour of the vapour was perceivable at the bottom as well as at the top. The quantity of nitrate found in these soils is so little different from that present in the soil before aspiration, that we may

safely conclude that in these two cases no nitrate, or at least an insignificant amount, has been produced in the course of the experiment.

The result of this branch of the investigation is, therefore, to prove that chloroform vapour will effectually prevent nitrification in a soil rich in vegetable matter, a fact directly confirmatory of Schløsing and Müntz's result obtained with sewage. We also learn that bisulphide of carbon is equally effective with chloroform, and that carbolic acid is probably effective to the extent in which it comes in contact with the soil. It thus appears that antiseptics, as a class, are inimical to nitrification; a result which is hard to explain if this process be one of simple oxidation only.

We turn now to the experiments made at Rothamsted upon the second branch of the inquiry. The object aimed at here was to ascertain if nitrification could be induced in a weak solution of chloride of ammonium, by seeding with a small quantity of a nitrifying body.

Four stoppered pint bottles of pale green glass were taken; each was half filled with the dilute solution of chloride of ammonium used in Nesslerising (1 c.c. = .00005 gram ammonia), to which a small quantity of acid phosphate of potassium was added. The object of the phosphate was to provide food for the organism to be presently introduced. Two of these bottles were placed in the window, and two in a dark cupboard. To one of the bottles in each series was added 1 c.c. of a mixture made by stirring together 1 gram of kitchen garden soil, and 5 c.c. of water. The soil used in this experiment was rich in nitric acid; it had, however, been kept for two or three years in an air-dry condition. The experiment commenced on May 19th.

On June 2nd all the bottles were tested, but no nitric acid found. Thinking that the slight acidity of the solution might be unfavourable to nitrification, a little freshly precipitated carbonate of calcium was at this date added to each bottle.

On July 11th the solutions were again tested, but no nitric acid found. In the bottle seeded with garden soil, and exposed to daylight, a film of green growth had commenced on the bottom of the bottle. Thinking that the solutions might be too strong, or that the germs of the garden soil had lost their vitality by long keeping, the bottles were now in every case filled with water to the shoulder, and the two bottles already seeded received in each case 1 gram of surface soil, obtained three weeks previously from a "fairy ring." It need hardly be stated that the "fairy-rings" found in meadows contain in the circle of luxuriant grass a more or less vigorous growth of fungi. A sample of the soil beneath the ring had in this case been taken to the depth of 12 inches; this sample contained in its upper part a small quantity of mycelium. The whole 12 inches was air-dried, crushed, and sifted, and from the fine soil 1 gram taken for each of the seeded bottles.

The bottles were next examined on October 11th. No nitric acid was found in either of the unseeded bottles. In the seeded bottle standing in daylight the green growth had increased. No nitric acid was found in this bottle; the application of the Nessler test proved that ammonia was still abundant. With the seeded bottle preserved in darkness the case was wholly different; here abundance of nitric acid was found,* amounting to 18·89 parts of nitrogen per million, while the Nessler test showed that ammonia had completely disappeared. The exact strength of the chloride of ammonium solution before nitrification was unfortunately not known, as at the previous examination it had been diluted with about its own volume of water. If the dilution had been exactly to twice its volume, it would have contained 20·58 parts of nitrogen per million.

We have here, then, a complete nitrification of an ammonium salt, brought about without any attempt to aërate the fluid, by the simple addition of a little soil from a fairy-ring, and of small quantities of phosphates, potash, and lime. The action also takes place exclusively in the dark, the perfectly similar mixture exposed to daylight exhibiting no nitrification.

It was evident that if the solution just obtained was really full of nitrifying organisms, it ought to be capable of acting itself as seed, and of setting up nitrification in other solutions. On October 15th the bottle containing the completely nitrified solution was shaken, and 1 c.c. of the contents added to each of the bottles—one in daylight, and the other in darkness—which had as yet remained unseeded.

On November 6th the solutions were examined. That in the window showed no nitric acid; that in the cupboard contained a trace, amounting to about 0·72 parts of nitrogen per million. Thinking that the nitrifying organism might require organic carbon for its growth, which these solutions did not supply, 50 milligrams of acid tartrate of potassium were added to each bottle.

On November 19th the solutions were again examined. That in daylight still showed no nitric acid. That in darkness now contained nitric acid equal to 2·04 of nitrogen per million.

On December 3rd the nitric acid in the solution kept in darkness had risen to 11·89 of nitrogen per million; but in the corresponding bottle in the window none was found. The experiment is still in progress.†

* The examination for nitric acid, and its quantitative determination, was made by means of indigo (*Chem. News*, xxxv, 45, 57). This method is admirably suited for such a purpose. 10 c.c. of the solution were sufficient for qualitative testing, and a few successive experiments with 10 c.c. sufficed for a quantitative determination.

† On December 19th the nitric acid had increased to 20·06 parts of nitrogen per million; a trace only of ammonia was present. The bottle in daylight still contained no nitric acid.

We have here, then, fresh proof that nitrification can be started in one solution from another, when without such seeding no nitrification would take place. The conclusions of Schlöesing and Müntz are thus again confirmed, with the addition of the important fact that darkness is apparently essential to the action of the nitrifying germs.

That the nitrification observed in soils and waters is due to the growth of some mycoderm, must now be considered as highly probable. The process thus becomes perfectly analogous to the acetic fermentation, in which alcohol is oxidised by the growth of *Mycoderma aceti*. The mycoderm producing nitrification has probably special characters, and might be sought for by a microscopical examination of the nitrified solutions already described. This part of the investigation must, however, be left to other observers.

POSTSCRIPT.—January 4th, 1878.—In a paper read before the Royal Society, on December 6th, it is shown by Messrs. A. Downes and T. P. Blunt, that light is inimical to the development of bacteria, a few hours' exposure to daylight being in many cases sufficient to destroy all the germs existing in an organic fluid, while similar solutions kept in darkness developed bacteria freely. The bearing of this observation on the fact already recorded as to the influence of light on nitrification is obvious. The influence of light on nitrification was not apparently quite unknown; it is twice hinted at in Gmelin's Chemistry (*Cavendish Society's Translation*, iii, 68; vii, 92), but is not mentioned by most writers on the subject.