

SOME NEW METHODS OF SOIL ANALYSIS*

By Dr. V. SUBRAHMANYAN, M.A., D. Sc.,

Indian Institute of Science, Bangalore.

The present communication relates to some new methods which have been recently developed in the laboratories of the Indian Institute of Science. They have been successfully applied in the study of biochemical problems relating to soils. They combine accuracy with rapidity and ease of handling and are capable of adoption in routine practice.

Carbonate, Organic Carbon and Total Nitrogen. In recent years, large number of methods have been developed for the estimation of these important constituents. Many of them are individually highly efficient, but none of them can be adopted for all the three determinations on the same sample. The method developed by Bhaskaran, Harihara Iyer and Rajagopalan fulfils that condition in addition to being extremely simple and rapid. The procedure may be outlined as follows:—

Carbonate. The soil (10 g.) is weighed out into a round bottomed flask (capacity, 300—500 c. c.), treated with 15 c. c. of water and the suspension well shaken. Syrupy phosphoric acid (sp. gr., 1·8; 10 c. c.) is introduced, from above, through a thistle funnel and the suspension raised to gentle boil. The vapours pass through a wide tube (about 8" long and 1" wide) which acts as an air condenser. Water vapour is cooled in the condenser and drops back into the distilling flask. Carbon dioxide passes over into an absorption system which consists of a water cooled straight condenser filled with glass beads, over which standard alkali (2N) is kept steadily trickling from above. The cooling combined with the large surface exposed by the glass beads facilitates quantitative absorption of the carbon dioxide.

After boiling for about 20 minutes, the heating is stopped and the alkali adhering to the glass beads washed down into a receiving flask. The unused alkali is titrated against standard acid (2N) after treatment with excess of barium chloride (10 per cent.; 20 c. c.). The end-point is sharpened by either finishing the titration against weaker acid (N/10) or back titrating against dilute alkali until the phenolphthalein colour is just regained.

Sulphuric acid is unsuitable for the above estimation because in presence of alkaline earth carbonates—which are present in many soils—the entire quantity is not decomposed even after prolonged boiling. The insoluble sulphate which is first formed, protects the

* A paper presented by Prof. V. Subrahmanyam (Indian Institute of Science, Bangalore) on behalf of Messrs. T. R. Bhaskaran, S. V. Govindarajan, C. R. Harihara Iyer, K. M. Pandalai and R. Rajagopalan before the Agricultural Section at the 23rd Session of the Indian Science Congress held at Indore (January 1936).

unattacked carbonate from reaction with the acid so that low and incorrect estimates are generally obtained. Hydrochloric acid is effective in decomposing the carbonate, but the residue is unsuitable for the estimation of organic carbon. On the other hand, phosphoric acid, if added in sufficient excess, decomposes all the carbonates (including those of alkaline earths) forming the soluble acid phosphate. The acid itself is nonvolatile and does not interfere with the subsequent estimations of organic carbon and total nitrogen.

Organic Carbon. To the residue after the estimation of carbonate, mercuric oxide (red or yellow, 2g.) is added, followed by treatment with concentrated sulphuric acid (nitrogen-free, 40 c. c.). The mixture is then just raised to boil and treated with chromic acid (sat. aq. solution, 5 c. c.) the latter being introduced through the funnel. (Care should be taken to see that none of the gases escapes through the funnel itself. If the pressure of the gas inside the flask is high, it may be necessary to blow in the chromic acid. A rubber tube extension provided with soda-lime guard is useful for the purpose). The boiling is then resumed and the water vapour together with traces of nitric acid which pass over condensed as before. Carbon di oxide is absorbed by the alkali trickling over the glass beads in the condenser. The unused alkali is titrated in the manner described above.

Total Nitrogen. The residue after digestion of organic carbon contains the entire quantity of nitrogen originally present in the soil. The major part is present as ammonium sulphate. Small proportions also occur (a) in combination with the chromium and the mercury in the digest and (b) as nitric acid, (in addition to any that may have been originally present as nitrate). The nitrogen in the former condition is released and that present in the latter reduced to ammonia by treatment with excess of alkali sulphite followed by boiling (5—10 minutes) with zinc (2 g.). (5—7 g. of pure sodium sulphite is usually required for the reduction. The sulphite need not be weighed, but may be added in small instalments until the colour changes from emerald green to a dirty blue. There will also be the characteristic separation of the black precipitate containing mercury. Zinc should be added after the mixture has been boiling for 1 or 2 mins. for, otherwise, there may be the undesirable formation of hydrogen sulphide). After cooling, the reduced digest is distilled with excess of alkali and the resulting ammonia estimated in the usual way.

Harihara Iyer and Rajagopalan have drawn attention to the loss of nitrogen in the elementary form if chromic acid is added to a cold or moderately hot mixture of the material to be digested, with sulphuric acid. This is due to the intermediary formation of ammonium dichromate, which, on further heating, undergoes spontaneous decomposition forming elementary nitrogen. (The decomposition occurs at between 120° and 150° C.). By adding chromic acid to the boiling mixture with

2:1 acid (temp. 170°) the formation of the intermediary compound and the subsequent loss of nitrogen are avoided.

It is well known that the presence of halides (generally chlorides) in the soil or any of the reagents causes the formation of the corresponding free halogen and consequent loss of nitrogen. This is prevented, however, by addition of a mercury salt (preferably the oxide). The efficiency of the added mercury salt varies with different soils, but ordinarily, interference of halides upto 2 per cent. can be prevented by the treatment.

The advantages of the foregoing method may be enumerated as follows. (1) The procedure is very simple and rapid. (2) The determinations are carried out on the same sample so that undue wastage of chemicals and apparatus is avoided. (3) Interfering substances are eliminated and the accuracy of the estimate greatly increased. Given a number of sets of apparatus, an average worker can carry out 10 to 12 determinations of all the three constituents in the course of a working day.

Nitrites and Nitrates. There are several methods for the estimation of these two important constituents. A few of them are volumetric methods and yield accurate estimates, but the majority are of the colorimetric type and are generally applicable for only small quantities. Of the two constituents, the nitrite is the more difficult to estimate, largely because of its instability in presence of air and other reacting substances. As the result of this, the nitrite contents of many soils and biological materials are generally under-estimated. It has been recently observed by Pandalai that the stability of the nitrite is largely determined by the reaction of the medium; that if the soil is first rendered slightly alkaline and then clarified to remove interfering organic matter, the resulting filtrate contains all the original nitrite. The procedure may be outlined as follows. The soil (wet or dry, 50 g.) is treated with sufficient quantity of slaked lime in the form of suspension until the medium is distinctly alkaline. It is then treated with solutions of copper sulphate (10 per cent., 5—10 c. c.) and basic lead acetate, (sp. gr., 1.24; 1—2 c. c.) and well shaken. This is followed by addition of a further quantity of slaked lime (with frequent shaking) until the supernatant is no longer blue, but is quite clear and colourless. The suspension is then filtered and washed frequently with water.

The filtrate is quite colourless and can be used as such for the two estimations. If there is even a faint colour, the clarification should be repeated until a clear, colourless extract is obtained. The extract is then divided into two portions one of which is used for the estimation of nitrite and the other, nitrate. If so desired, the same sample of extract can also be used for both the estimations.

Nitrite. The extract is treated with excess of standard permanganate followed by addition of dilute sulphuric acid. The mixture is well shaken and the unused permanganate titrated either directly against ferrous ammonium sulphate or indirectly (after addition of potassium iodide) against standard thiosulphate.

Direct estimation of nitrite is rendered possible by the efficient clarification which removes all interfering substances. Nitrite is the only reducing agent in the medium so that the quantity of permanganate used up gives the correct estimate of that form of nitrogen.

The estimation can also be conducted after addition of excess of standard hydrogen peroxide solution, the unused part being subsequently titrated against standard permanganate. Hydrogen peroxide is comparatively stable in dilute acid solutions, but the strength of the reagent will, nevertheless, have to be checked from time to time. On the other hand, direct titration of the acidified extract against permanganate (from the burette) yields inaccurate estimates. Nitrous vapours (NO and NO_2) are partly lost from the system so that the resulting values are generally low. Nitrites do not react in the cold with dichromate, so that it is necessary to heat the acidified solution with excess of dichromate ($60-80^\circ$). Even, then, the reaction may not always be complete.

Nitrates. A further portion of the original extract is treated with slight excess of alkaline permanganate and boiled for about 15 minutes. Ammonia is removed and traces of albuminoids, if any, oxidised and driven out as ammonia. The residue, which contains only the nitrate (together with the nitrite) is then distilled with Devarda's alloy in the usual way.

An interesting feature about the foregoing estimation is the effect of minute quantities of lead (or any other heavy metal) on the smoothness of reduction with Devarda's alloy. Ordinarily, reduction with Devarda's alloy or aluminium in alkaline medium proceeds very vigorously with the result that minute quantities of alkali are often mechanically carried over with the spray. This necessitates redistillation. On the other hand, presence of minute quantities of heavy metals leads to light protective coats of those metals (or their hydrides) being found around the reducing agent so that the reduction proceeds smoothly and quantitatively. Excess of metallic salts should be avoided, for, the reaction is then so much retarded that the reduction is not complete even after several hours. In the present method, the small quantity of lead passing into the filtrate is just sufficient to render the reaction smooth and quantitative.

The foregoing method has been utilised for the determination of nitrites and nitrates in a number of tropical soils. It is also eminently suitable for the study of the changes in different seasons and at various stages both before and after manuring. The transformations in

the swamp soils, especially in the earlier stages of puddling (when the conditions are favourable for reduction of the nitrite) will also be of much practical value.

Manganese. Among the various methods for the estimation of manganese, those involving the use of bismuthate are the most rapid and, in many respects, the most convenient. The procedure consists in first extracting the manganese and then converting it into permanganate, which can be quantitatively estimated. The actual working is, however, more difficult and often less accurate than is generally recognised. In the first place, many of the commoner reagents used for the extraction—hydrogen peroxide, ferrous sulphate, zinc, reduced iron, oxalic acid and such like—do not extract quantitatively, so that a small portion of the manganese is always left behind. Secondly, the extractants themselves react with the bismuthate thus involving considerable wastage of that rather costly chemical. There is also interference from extracted organic matter, which, if present in more than minute quantities, leads to low estimates of manganese being obtained. These and other defects are obviated in the procedure developed by Harihara Iyer and Rajagopalan which may be described as follows:—The soil (10 g. or more) together with mercuric oxide (2 g.) is weighed into a conical flask (cap., 250 c. c.) and treated with excess of 2–4 N sulphuric acid, followed by pure sodium sulphite (5 g.). After the vigorous initial reaction has subsided, the suspension is raised to boil and excess of sulphite driven off as sulphur dioxide. The soil suspension is then filtered and the extract treated with sodium bismuthate (0.5 to 1.0 g.) and raised to boil. After boiling for about 1 min., the flask is momentarily taken out to see if the suspension has assumed a definite pink colour. If the colour is either not formed, or disappears rapidly after formation, more bismuthate is added (0.5 g. at a time) followed by further heating. When the colour is finally established, the suspension is filtered through gooch or Jena glass filter. The filtrate is treated with excess of ferrous ammonium sulphate and the unused portion back titrated against standard potassium permanganate in the usual way.

Chlorides interfere with the accuracy of the estimation. On treatment with bismuthate, free chlorine is formed to some extent and reacts with ferrous ammonium sulphate, thus giving exaggerated estimates of manganese. This defect is eliminated by the addition of the mercury salt (preferably the oxide) which prevents the formation of chlorine. Another source of error is the bismuthate itself which contains minute quantities of some water soluble constituent which reacts with ferrous ammonium sulphate. The error due to this, is however, generally small, being the order of 0.1 to 0.2 c. c. of N/10 solution. It is proportionate to the quantity of bismuthate used so that the necessary correction can be applied by using weighed quantities of that reagent.

The foregoing method has been successfully applied to the estimation of manganese in a large number of tropical soils. The procedure is also adapted for the study of the transformations of manganese under different conditions in dry as well as wet cultivated soils. The method can be extended to the estimation of manganese in plant and animal products after destroying the organic matter by some suitable method.

Aluminium. Although this element is one of the most abundant of soil constituents, yet it is mostly present in the insoluble form and generally inactive in the ordinary type of soils. Under certain abnormal conditions however—such as high acidity or alkalinity—its solubility is increased with the result that certain adverse effects are observed. It is yet difficult to state whether the toxicity to plants is directly traceable to the aluminium ion, but there is no doubt that its presence in abnormally large quantities often indicates such a condition.

The older methods for the estimation of aluminium in soils and biological materials are elaborate and tedious. The discovery of the new reagent, 8—hydroxyquinoline (oxine), has, however, greatly simplified the procedure and increased the accuracy of the estimation. Oxine is, not however, specific to aluminium so that care has to be taken to remove iron and other heavy metals which are generally associated with the soil. Silicon and organic matter interfere with the precipitation of the aluminium salt and must also be removed. The modified procedure developed by Govindarajan may be outlined as follows.—The material (soil, 1-2 g.) is ignited at low red heat for 2-3 hours. It is then cooled and extracted with alkali (2—4 N). The extraction must be repeated a few times so as to facilitate all the aluminium passing into solution. (Heating with alkali is undesirable, as it will increase the solubility of silica). The extract is treated with a solution of a ferric salt, (sulphate or chloride. 0.5 g.) and the resulting suspension just raised to boil. It is then cooled and filtered, a suspension of iron hydroxide in alkali being used for washing. The filtrate is rendered faintly acid and treated with a solution of oxine in acetic acid. The mixture is warmed to 70° to facilitate the precipitation of the aluminium compound and then filtered followed by washing with warm water. The precipitate is dissolved in excess of hydrochloric acid (20—30 c. c. ; 1 : 4) and titrated against bromide-bromate mixture (N/10) with indigo carmine as indicator. Alternatively, excess of the bromide-bromate mixture may be added and the unused portion determined by addition of potassium iodide followed by titration against standard thiosulphate.

The novel features in the foregoing method are that (1) the organic matter is first destroyed by ignition and (2) silicon is removed by adsorption with ferric hydroxide. The method has been successfully

fully applied for the estimation of aluminium in soils and a variety of biological materials. It has also been extended to a study of the distribution of aluminium under different conditions in the swamp soil.

Summary. (1) Elegant and rapid methods for the estimation of some important soil constituents—carbonate, organic carbon, total nitrogen (including nitrate), nitrite, nitrate, manganese and aluminium—have been described.

(2) Estimation of carbonate, organic carbon and total nitrogen (including nitrate) can be carried out on the same sample. The carbonate is first decomposed by excess of phosphoric acid and the resulting carbon dioxide absorbed in a flowing stream of alkali in a condenser filled with glass beads. The residue can be used for the wet combustion of organic carbon, chromic acid being used for the purpose. By adding the oxidising agent to a boiling mixture of the soil with 2:1 sulphuric acid, intermediary formation of ammonium dichromate and consequent loss of nitrogen are avoided. The residue contains the entire quantity of the original nitrogen mostly as ammonium sulphate, but small portions also exist (a) in combination with mercury and chromium and (2) as nitric acid. The nitrogen in the two latter forms is converted to ammonia by treatment with alkali sulphite followed by boiling with zinc. The digest is then cooled and distilled with excess of alkali in the usual way.

(3) A direct volumetric method for the estimation of nitrite and nitrate in the same sample has been described. By first treating the soil with a small quantity of slaked lime, the nitrite is first stabilised. By adding a clarifying mixture consisting of copper sulphate and lead acetate together with excess of slaked lime, organic matter and other interfering substances are removed. The nitrite in the filtrate is estimated by addition of excess of permanganate or hydrogen peroxide and back titrating the unused portion. Nitrate is determined by boiling with alkaline permanganate followed by distillation with Devarda's alloy.

(4) Manganese is extracted by treatment with sulphite in acid medium. The extract is then treated with bismuthate in presence of a mercury salt (preferably the oxide) and the resulting permanganate determined in the usual way.

(5) For the estimation of aluminium, the organic matter of the soil is first destroyed by ignition at a low red heat. The residue is extracted repeatedly with alkali. The extract is treated with an iron salt (to absorb the silica) and the resulting suspension filtered. The filtrate is treated with hydroxyquinoline (oxine) reagent in a faintly acid medium. The resulting precipitate is dissolved in hydrochloric acid and titrated against bromide-bromate mixture.

Acknowledgements are due to Messrs. T. R. Bhaskaran, S. V. Govindarajan, C. R. Harihara Iyer, K. M. Pandalai and R. Rajagopalan who were mainly responsible for the researches described in the paper.

The foregoing method has been successfully applied to the estimation of manganese in a large number of tropical soils. The procedure is also adapted for the study of the transformations of manganese under different conditions in dry as well as wet cultivated soils. The method can be extended to the estimation of manganese in plant and animal products after destroying the organic matter by some suitable method.

Aluminium. Although this element is one of the most abundant of soil constituents, yet it is mostly present in the insoluble form and generally inactive in the ordinary type of soils. Under certain abnormal conditions however—such as high acidity or alkalinity—its solubility is increased with the result that certain adverse effects are observed. It is yet difficult to state whether the toxicity to plants is directly traceable to the aluminium ion, but there is no doubt that its presence in abnormally large quantities often indicates such a condition.

The older methods for the estimation of aluminium in soils and biological materials are elaborate and tedious. The discovery of the new reagent, 8—hydroxyquinoline (oxine), has, however, greatly simplified the procedure and increased the accuracy of the estimation. Oxine is, not however, specific to aluminium so that care has to be taken to remove iron and other heavy metals which are generally associated with the soil. Silicon and organic matter interfere with the precipitation of the aluminium salt and must also be removed. The modified procedure developed by Govindarajan may be outlined as follows.—The material (soil, 1-2 g.) is ignited at low red heat for 2-3 hours. It is then cooled and extracted with alkali (2-4 N). The extraction must be repeated a few times so as to facilitate all the aluminium passing into solution. (Heating with alkali is undesirable, as it will increase the solubility of silica). The extract is treated with a solution of a ferric salt, (sulphate or chloride. 0.5 g.) and the resulting suspension just raised to boil. It is then cooled and filtered, a suspension of iron hydroxide in alkali being used for washing. The filtrate is rendered faintly acid and treated with a solution of oxine in acetic acid. The mixture is warmed to 70° to facilitate the precipitation of the aluminium compound and then filtered followed by washing with warm water. The precipitate is dissolved in excess of hydrochloric acid (20-30 c.c.; 1:4) and titrated against bromide-bromate mixture (N/10) with indigo carmine as indicator. Alternatively, excess of the bromide-bromate mixture may be added and the unused portion determined by addition of potassium iodide followed by titration against standard thiosulphate.

The novel features in the foregoing method are that (1) the organic matter is first destroyed by ignition and (2) silicon is removed by adsorption with ferric hydroxide. The method has been successfully

fully applied for the estimation of aluminium in soils and a variety of biological materials. It has also been extended to a study of the distribution of aluminium under different conditions in the swamp soil.

Summary. (1) Elegant and rapid methods for the estimation of some important soil constituents—carbonate, organic carbon, total nitrogen (including nitrate), nitrite, nitrate, manganese and aluminium—have been described.

(2) Estimation of carbonate, organic carbon and total nitrogen (including nitrate) can be carried out on the same sample. The carbonate is first decomposed by excess of phosphoric acid and the resulting carbon dioxide absorbed in a flowing stream of alkali in a condenser filled with glass beads. The residue can be used for the wet combustion of organic carbon, chromic acid being used for the purpose. By adding the oxidising agent to a boiling mixture of the soil with 2:1 sulphuric acid, intermediary formation of ammonium dichromate and consequent loss of nitrogen are avoided. The residue contains the entire quantity of the original nitrogen mostly as ammonium sulphate, but small portions also exist (a) in combination with mercury and chromium and (2) as nitric acid. The nitrogen in the two latter forms is converted to ammonia by treatment with alkali sulphite followed by boiling with zinc. The digest is then cooled and distilled with excess of alkali in the usual way.

(3) A direct volumetric method for the estimation of nitrite and nitrate in the same sample has been described. By first treating the soil with a small quantity of slaked lime, the nitrite is first stabilised. By adding a clarifying mixture consisting of copper sulphate and lead acetate together with excess of slaked lime, organic matter and other interfering substances are removed. The nitrite in the filtrate is estimated by addition of excess of permanganate or hydrogen peroxide and back titrating the unused portion. Nitrate is determined by boiling with alkaline permanganate followed by distillation with Devarda's alloy.

(4) Manganese is extracted by treatment with sulphite in acid medium. The extract is then treated with bismuthate in presence of a mercury salt (preferably the oxide) and the resulting permanganate determined in the usual way.

(5) For the estimation of aluminium, the organic matter of the soil is first destroyed by ignition at a low red heat. The residue is extracted repeatedly with alkali. The extract is treated with an iron salt (to absorb the silica) and the resulting suspension filtered. The filtrate is treated with hydroxyquinoline (oxine) reagent in a faintly acid medium. The resulting precipitate is dissolved in hydrochloric acid and titrated against bromide-bromate mixture.

Acknowledgements are due to Messrs. T. R. Bhaskaran, S. V. Govindarajan, C. R. Harihara Iyer, K. M. Pandalai and R. Rajagopalan who were mainly responsible for the researches described in the paper.