Quick-tests for soil and plant analysis used by small laboratories

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Summary

A description is given of a number of quick and simple methods adapted for use in small laboratories for the analysis of soil and plant samples. It is shown that with the methods described a satisfactory degree of accuracy and reproducibility is obtained under conditions prevailing in the Netherlands.

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1. Introduction

Research on soil and plants performed in small laboratories has features of its own. Its main purpose is the orientation of the research worker with respect to the nature of the problems concerned. A great diversity in analytical methods is therefore required. Although these methods need not be too accurate they should nevertheless be accurate enough to allow this orientation, this qualification of his problems.

The research worker has usually at his disposal a small laboratory with one or two assistants. The nature of the work calls for a flexible organisation which could handle one determination or another at a moments notice. It is therefore impossible to draw an extended work schedule for the benefit of the assistants. Moreover, all the work in connection with the reception, the preparation of the samples (drying, grinding, sieving, extracting and digesting), the analysis and drafting of the results has to be carried out by the same person(s).

It is therefore desirable to choose simple and specially quick tests. From beginning to end the determination should not take more than a few hours.

The inconveniences due to lack of space in small laboratories should also be stressed. As long as benches are occupied, other determinations have to be postponed. Hence chemical separations should be avoided. Moreover, this refinement is often rather time consuming. It is advisable to use as few different extractions or digestions as possible for the determination of the elements considered.

All the above mentioned considerations lead to the use of micro- or semimicro methods. Apart from a more economic use of working space and chemicals, simpler glassware can be used (test tubes instead of graduated flasks or erlenmeyer flasks), the cleaning of which is less laborious; finally, use can be made more advantageously of automatic devices.

Because of the qualitative nature of the work determinations do not need to be too accurate. Depending on the element under consideration, the type and use made of the soil and the time of sampling, the variation coefficient of the sample will be about 15 % (FERRARI and VERMEULEN, 1955). Therefore time consuming manipulations to improve accuracy of determinations are in many cases illogical. It seemed therefore advisable to use as a starting point for the development of analytical methods applicable to analysis of soils and plant material in small laboratories the work of MORGAN (MORGAN, 1935, 1937; LUNT, SWANSON and JACOBSON, 1950) and of LINDNER and HARLEY (LINDNER and HARLEY, 1942; LINDNER, 1944).

MORGAN's article on his "Universal Soil Testing System" created great interest in quick tests for soil sample analysis. In the former Dutch East Indies, VENEMA (VENEMA, 1943 a and b, 1944/45) employed with success modifications of MORGAN's methods for the classification of a great number of soil samples.

The basic principle of MORGAN's method is that in order to reach a correct evaluation of the soil fertility it is necessary to consider together as many properties as possible since it is the interaction of these properties which determines the quality of the soil (SCHUFFELEN, 1948). This principle is of great importance, particularly in preliminary research.

In order to apply it, it is necessary to make quick tests all based preferably on the same extraction or digestion method.

For this purpose MORGAN chose a Na-acetate-acetic-acid buffer solution of pH 4,8 since an acetate buffer has the maximum buffering capacity at this pH.

Since 1937 attempts have been made at the Laboratory of Soils and Fertilizers at Wage-

ningen to adapt these "quick tests" to Dutch soils. In addition to MORGAN's original analysis specifications this work was also based on the reports of VENEMA (1943 a and b, 1944/45), PEECH and ENGLISH (1944) and SNELL and SNELL (1949).

For the analysis of plant material, the methods used are based on the work of LINDNER and HARLEY (LINDNER and HARLEY, 1942; LINDNER, 1944) who used a sulfuric acid-hydrogen peroxide digestion in which both N and the inorganic constituents (Na, K, Ca, Mg and P) could be determined.

It is not always possible to keep the work as simple as desirable. In addition to the analysis carried out in a MORGAN's extract other determinations had to be introduced in order to allow a better interpretation of the data. An attempt was therefore made to reach a reasonable compromise between speed, simplicity, accuracy and reproducibility.

About 1950 this work was continued in conjunction with regional horticultural laboratories (Research Station for Floriculture at Aalsmeer; Research Station for vegetable growing in the open at Wilhelminadorp; Research Station for flower bulb growing at Lisse; Research Station for fruit and vegetable growing under glass at Naaldwijk and the Government Advisory Services at Kesteren and Maastricht) and a number of private and semi-private laboratories (Netherlands Agricultural Lime Foundation at de Bilt; Potash Importing Cy at Amsterdam; Laboratory for Soil and Crop Testing at Oosterbeek; Royal Institute for the Tropics at Amsterdam and the Syndicat pour l'amélioration des sols et des cultures at Gargenville, France).

This joint work on the improvement of methods and interpretation of results is still in progress. For this purpose soil and plant samples are distributed monthly. The data obtained from this check are analyzed to determine among other things, the coefficients of variation. In TABLE 1 these coefficients are compared for a number of years. It is apparent that improvements made in the analytical methods have been successful and that a standard has been reached in which the methods are sufficiently accurate and reproducible. Because during 1959 the methods were further improved the coefficients of variation are actually lower.

TABLE 1. Coefficient of variation relating to the results of 11 laboratories during 1950, 1957 and 1958.

Soil analysis						Plant analysis			
Determination	ppm range	Content on which coeff. of variation is based	Coefficient of variation 1950	1957	1958	ppm range	Content on which coeff. of variation is based	Coefficient of variation 1957	1958
K	1— 200	60	35	9	9	20—140	70	6	5
P	1— 90	15	20	10	10	(PO_4)1— 20	12	10	10
Mg	10— 300	68	45	10	10	1— 26	10	15	15
Fe	1— 12	7	60	15	12				
Mn	1— 25	6.6	70	15	20				
NO_3	1— 30	6.6	70	19	15				
Ca	50—5000	2600	35	5	5	10—140	20	17	8
Al	1— 30	3	70		30				
Al		10			15				
N						50—300	135	5	3
Na						1— 28	10		12

2. Analysis of soil and plant samples

In the analytical methods given, use is frequently made of colorimetric reactions. In order to compare the colours obtained in samples with those of the standard series a visual comparator [1] is required. In the magnesium determination of digested plant samples, the blank is so highly coloured that it is difficult to judge visually. In this case it is desirable to use a simple colorimeter. In quick tests it is customary to determine the K or Ca content by means of turbidity reactions. These proved to be so unreliable (cf. TABLE 1 soil analyses, K and Ca 1950) that flame photometric determinations of these elements was adopted instead. This method was also found to be cheaper.

pH measurements are carried out using a glass electrode standard pM meter, and a conductometer is employed for the determination of the total salts in the soil extract (residue on ignition (extract)).

For Kjeldahl nitrogen determination use is made of a microdistillation apparatus of one of the authors' own design (v. S.). Automatic pipettes are used as much as possible for the addition of reagents.

2.1. Soil samples

The research programme of the regional laboratories includes determination of pH-water, pH-KCl, residue-on-ignition (extract), Cl, Fe, Al, Ca, Mg, K, Mn, P, N-NO_3 and N-NH_4 as well as the determination of reducible manganese. In all cases the starting material is well mixed and air-dry soil which has been screened through a 2 mm sieve.

2.1.1. *Determination of pH in water suspension*

Procedure. 20 g of soil are shaken with 50 ml of distilled water for a minute by hand. The samples are shaken again by hand 3 hours later. The suspension is allowed to stand overnight, the samples are shaken a third time, and the pH is determined immediately afterwards in the supernatant solution (glass electrode, calomel reference electrode).

2.1.2. *Determination of pH in KCl solution*

Procedure. The procedure is identical to that described for pH-water, except that 1 N KCl is used instead of distilled water.

2.1.3. *Determination of the residue-on-ignition (extract)*

Principle. An empirical relationship exists between the ohmic resistance of a filtrate and the residue-on-ignition obtained following evaporation to dryness and ignition (ANON., 1960). This relationship depends on the organic matter content of the soil; in the case of soils having a humus content (loss-on-ignition) of less than 15 %, it may be approximately represented by the equation:

$$\frac{442}{\text{resistance at } 18°} = \% \text{ residue-on-ignition,}$$

in the case of soils having a humus content (loss-on-ignition) equal to or greater than 15 %, the equation becomes:

$$\frac{518}{\text{resistance at } 18°} = \% \text{ residue-on-ignition.}$$

[1] A simple device made of a strong light source behind a milky glass screen allowing visual comparison of the sample with a series of individual standards.

Procedure. 25 g of soil are shaken with 125 ml of distilled water as described for the pH determinations. After the third shaking the suspension is filtered (Delta 314 3/4); filtration is repeated through the same filter if filtrate is turbid. The temperature, and the resistance (by means of a conductometer) of the filtrate are determined. After the resistance found has been converted to the specific resistance at 18° the percentage residue-on-ignition can be traced back in a table based on the formulae given.

2.1.4. *Determination of the chloride content*

Principle. $HgCl_2$ is so slightly ionised in water that the mercuric ion does not colour diphenylcarbazone. This enables the chloride ion to be titrated with $Hg(NO_3)_2$ (DOMASK and KOBE, 1952).

Reagents. 1. $Hg(NO_3)_2$, 0,02 N : dissolve 8,7 g of $Hg(NO_3)_2$ and 6 ml of HNO_3(60 %), make up to 2,5 litres with distilled water. Standardise against NaCl; 2. HNO_3 0,2 N; 3. Mixed indicator stock solution: dissolve 0,5 g of diphenylcarbazone and 0,05 g of bromophenol blue separately in 50 ml of warm ethanol 96 %. Mix the two solutions; 4. Mixed indicator working solution: add together equal volumes of mixed indicator stock solution, HNO_3 0,2 N and distilled water. A precipitate is formed which should not be filtered off; the turbid solution is used as reagent.

Procedure. To 25 ml of the extract obtained in the residue-on-ignition determination (method 2.1.3.) add 1 ml of mixed indicator working solution. Add sufficient HNO_3 0,2 N for the extract to turn yellow (pH 3—3,5; in this pH range $HgCl_2$ is formed). Then titrate with $Hg(NO_3)_2$ 0,02 N until the purple colour of the blank determination is obtained.

Calculation. ml ($Hg(NO_3)_2$ (after allowing for the blank determination) $\times$ titre $Hg(NO_3)_2 \times 1{,}17 =$ % NaCl. The result is calculated assuming all chlorides are derived from NaCl.

2.1.5. *Determinations in the Morgan extract*

Morgan soil extracts are often coloured by dissolved organic compounds. In colorimetric determinations this colour may have an interfering effect. Peech and English therefore added activated charcoal to the extract. Our own investigations showed that the addition of different types of charcoal can change the composition of the extract, depending on the type of coal used (VAN SCHOUWENBURG, 1959). This effect is particularly serious with the trivalent cations, so that Fe and Al determinations should be made taking into consideration the extract's own colour. Ca and K are determined without objection by flame photometry in the non-decolorised extract. After Fe, Al, K and Ca have been determined the extract can be treated with activated charcoal; Norit PX and Medicinal Norit (in powder form) are particularly suitable for this purpose (0,1 g/10 ml extract). Owing to the mutual interferences Mn, Mg, P, $N\text{-}NO_3$ and $N\text{-}NH_4$ are determined successively in the now colourless filtrate.

In the analyses efforts were made to minimise the effect of interfering ions. This could only be partially achieved, and interferences may still occur above a limiting concentration, specified in the directions.

For duplicate determinations we therefore recommend the use of another dilution of the extract. If the results do not correspond to the dilution used the presence of an interfering ion may be expected. By further diluting the extract it is usually possible to decrease the concentration of the interfering ion below the limiting level.

Previous separation is necessary when there exists a very unfavourable ratio of the

ion to be determined to the interfering ion. This separation need not be quantitative. Such conditions, however, are practically unknown in Dutch soils.
In order to counteract as far as possible the disturbing effects of time and temperature a standard series should be run with each series.
In the case of methods largely dependent on pH (Al determination) the $CaCO_3$ content of the soil should be taken into account. The buffering action of the Morgan extracting solution is sufficient only for soils having not more than 1,5 % $CaCO_3$. When the carbonate content is higher more acid should be added to reduce pH to the original 4,8 value.

The extraction. 20 g of air-dry soil are mechanically shaken for 30 minutes with 50 ml of extracting solution (100 g of sodium acetate + 30 ml of glacial acetic acid, diluted to 1 litre with distilled water; pH 4,8). A blank (without soil) should be included since both the filter and the Norit may contain contaminations. The soil solution is then filtered (Delta 314 3/4).
The extraction ratio is chosen considering the need for sufficient extract for all determinations, and the desirability of obtaining the highest possible concentration of ions in the extract. After Fe, Al, Ca and K have been determined in the extract, Norit PX or Medicinal Norit is added to it, about 0,1 g (judged visually) being added for each 10 ml of extract. The extract is then shaken by hand for a few minutes and filtered after an interval of about 5 minutes. The colourless filtrate is used for the other determinations.
Unless otherwise specified, in carrying out the determination the solution should be shaken after each addition of reagents in order to ensure thorough mixing. The standard series is invariably obtained by pipetting off increasing amounts of the standard solution (to 2 ml) and these are made up to 2 ml with Morgan extracting solution, (except for NH_4, reducible manganese and Al determinations).
The standard series is then treated in the same way as the unknown extracts.
A number of interferences are specified for each determination. This list is not complete. The unspecified sources of interference do not occur with any significant effect in Morgan soil extracts. All determinations are carried out in test tubes. Unless otherwise specified extracts should be diluted with Morgan extracting solution.

2.1.5.1. *Determination of iron.* Principle: Fe^{2+} yields a very stable orange-red complex with orthophenantroline.

Reagents. 1. standard solution 10 ppm of Fe: dissolve 0,070 g of $FeSO_4(NH_4)_2SO_4.6aq$ and 4 ml of hydroxylamine-HCl 5 %, dilute to 1 litre with Morgan extracting solution; 2. hydroxylamine-HCl 5 % in distilled water; 3. orthophenantroline 0,5 % in ethanol 96 %; 4. Mixed reagent: 10 ml of orthophenantroline 0,5 % made up to 250 ml with Morgan extracting solution.

Procedure. To 2 ml of extract, diluted if necessary, add successively:

1. 0,2 ml of hydroxylamine-HCl. Since only divalent iron reacts with orthophenantroline reduction with hydroxylamine-HCl is necessary. By the introduction or non introduction of distilled water instead of the reducing agent it is possible to distinguish between di- and trivalent Fe-ions.
2. After 1 minute add 2 ml of mixed reagent. The colour development is independent of the acidity between pH 2—9.

Measurement. After 10 minutes the colour is constant. In view of the extract's own colour it is advisable to compare the solutions visually with the standard

series, so as to make allowance for the extract's own colour. When a colorimeter is used read at 520 mμ.

Interferences. 1. Cu above 20 ppm; 2. P above 50 ppm; 3. SANDELL (1959) reports also Ag, Bi, Ni, Co, Cd, Hg, Zn, W and Sn.

2.1.5.2. *Determination of aluminium.* Principle: Al ions form with aluminon a coloured, inner complex salt. For both the standard series and the unknown extracts the determination is made with a volume of 1 ml of either standard or unknown. The pH of the extracts should be maintained at 4,8.

Reagents. 1. Standard solution 10 ppm of Al: 0,176 g of $K_2SO_4Al_2(SO_4)_3.24aq$ per litre of Morgan extracting solution; 2. Aluminon 0,2 % indistilled water; 3. hydroxylamine-HCl 5 % in distilled water; 4. Carbocel 0,5 % in distilled water [1]; 5. Carbocel (1 : 3) : dilute 1 part of Carbocel 0,5 % with 3 parts of Morgan extracting solution; 6. Mixed reagent: 10 ml of aluminon 0,2 % + 240 ml of Carbocel (1 : 3).

Procedure. To 1 ml of extract, diluted if necessary, add successively:

1. 0,5 ml of hydroxylamine-HCl 5 %. The iron present is reduced to ferrous ions. These interfere less than ferric ions.
2. After an interval of 1 minute add 5 ml of mixed reagent. The Carbocel acts as a protecting colloid.

Measurement. Measurements can be taken after 1 hour. In view of the extract's own colour it is advisable to compare the solutions visually with the standard series, so as to make allowance for the extract's own colour. When a solorimeter is used read at 545 mμ.

Interferences. 1. If the sample contains more than 1,5 % $CaCO_3$ the pH should be lowered with acid to 4,8; 2. Fe 5 ppm; 3. Cu 10 ppm; 4. P 1000 ppm; 5. SiO_2 500 ppm; 6. SANDELL (1959) reports also Ni.

2.1.5.3. *Determination of calcium.* The turbidity reaction with a soap reagent being unsatisfactory a flame photometric determination worked out for the Kipp flame photometer was successfully used instead. 1 ml of extract is diluted with 9 ml of distilled water to counteract the disturbing effect of Na in the Morgan extracting solution. 2,5 ml of $MgCl_2$ 5,00 N are then added. This reduces sufficiently the interference from any P, Al or SO_4 present. The solution is compared with a Ca standard series in the same medium (1 : 9 Morgan solution and $MgCl_2$ 1 N).

2.1.5.4. *Determination of potassium.* The turbidity reaction with cobalt nitrite was found unsatisfactory and a flame photometric determination was used instead. 1 ml of extract is diluted with 9 ml of distilled water. The solution is compared with a K standard series which also has a 1 : 9 Morgan medium.

After these determinations the extract is shaken with PX or Medicinal Norit (about 0,1 g of Norit to 10 ml of extract) and filtered. After this Norit treatment most of the Fe and Al have been adsorbed from the extract so that they cause practically no further interferences.

2.1.5.5. *Determination af manganese with periodate.* Principle: Potassium periodate oxidizes the manganese ion to permanganate.

Reagents: 1. Standard solution 20 ppm of Mn: 9,12 me of $KMnO_4$ are diluted with 500 ml of distilled water and 40 ml of concentrated H_2SO_4. A sufficient

1 Sodium carboxymethyl cellulose Z.L.C.F. ("NIJMA", Nijmegen, Netherlands).

amount of $NaHSO_3$ 10 % (in distilled water) is added dropwise, about 3 ml, until disappearance of the purple colour. The excess bisulphite is removed with 5 drops of HNO_3 65 %. This solution made up to 1 litre contains 100 ppm of Mn. Dilute to 20 ppm Mn in a Morgan medium; 2. KIO_4 1 % : dissolve in distilled water while heating; 3. H_3PO_4 8,5 % in distilled water; 4. HNO_3 (s.g. 1,40) dilute with distilled water, 1 : 4; 5. Mixed reagent: mix 100 ml of KIO_4 1 %, 20 ml of HNO_3 1 : 4, 40 ml of H_3PO_4 8,5 % and 300 ml of Morgan solution.

Procedure. To 2 ml of the extract, diluted if necessary, add 5 ml of mixed reagent. The H_3PO_4 allows the formation of a Fe complex and H_3PO_4 and HNO_3 together counteract the formation of manganese peroxide.

Measurement. After 20 minutes the colour remains constant for a conciderable time. When a colorimeter is used read at 540 $m\mu$.

Interferences. 1. Al 20 ppm; 2. Fe 20 ppm; 3. Reducing agents; 4. SANDELL (1959) reports Bi and Sn precipitates.

2.1.5.6. *Determination of manganese with formaldoxime.* Principle: In an alkaline medium manganese yields a reddish-brown compound with formaldoxime.

Reagents. 1. Standard solution 10 ppm Mn: see 2.1.5.5., but use half the Mn concentration; 2. Formaldoxime reagent: dissolve 2 g of hydroxylamine-HCl and 1 ml of formaldehyde 40 %, dilute to 50 ml with distilled water; 3. ascorbic acid 2 % in distilled water (can be kept for two days); 4. KCN solution: dissolve 40 g of NaOH and 40 g of KCN, dilute to 1 litre with distilled water; 5. EDTA.

Procedure. To 2 ml of extract, diluted if necessary, add successively:

1. 1 ml of ascorbic acid 2 %. For reduction of the ferric ion.
2. Two drops of formaldoxime reagent. The formaldoxime reagent cannot be kept in the dilute state, so that this addition should be followed immediately by the addition of:
3. 2 ml of KCN solution. The KCN serves to complex Fe, Co, Cu and Ni which yield coloured compounds with formaldoxime. The ferric-KCN complex is unstable in the presence of formaldoxime. Unlike the ferro-KCN complex, the ferric ion continues to interfere with the determination.
4. If a precipitate is formed, solid EDTA should be added after 30 minutes until the turbidity just disappears. A large excess of EDTA interferes, probably owing to complexing with the Mn.

Measurement. The measurements can be taken immediately after the addition of the EDTA. The colour remains constant for $2^1/_2$ hours. When a colorimeter is used read at 450 $m\mu$.

Interferences. 1. Fe 100 ppm; 2. Co 100 ppm; 3. Cu 10 ppm; 4. Ni 50 ppm; 5. Ca 500 ppm (without addition of EDTA).

2.1.5.7. *Determination of magnesium.* Principle: Thyazol yellow is coloured red by $Mg(OH)_2$.

Reagents. 1. Standard solution 20 ppm Mg: dissolve 0,203 g of $MgSO_4$. 7ag. in Morgan extracting solution, make up to 1 litre; 2. thyazol yellow "Geigy" 0,1 % in distilled water; 3. Carbocel 0,5 % in distilled water; 4. 200 ppm of Mn: dissolve 0,411 g of $MnSO_4$ 4 aq. in Morgan extracting solution, make up to 500 ml; 5. NaOH 5 N; 6. 200 ppm Mg in Morgan extracting solution; 7. Mixed reagent: add together 75 ml of glycerine (s.g. 1,23), 25 ml of Carbocel 0,5 %, 40 ml of thyazol yellow 0,1 %, 8 ml 200 ppm of Mn and 2 ml 200 ppm of Mg (this reagent can be used up to three days after its preparation).

Procedure. To 2 ml of extract, diluted if necessary, add successively:

1. 1,5 ml of mixed reagent. The glycerine and Carbocel serve to prevent precipitation of the lake. The manganese interference is independent of concentration between 20 and 50 ppm Mn. The amount of Mn added serves to bring the concentration of all samples to within this range. If no Mg is added it is impossible to determine 0—2 ppm Mg. The Mg in the mixed reagent is used for giving the standard series (and the samples) a concentration of at least 2 ppm.
2. 1 ml of NaOH 5 N. Store in a dark place.

Measurement. Measurements can be made after 45 minutes; the colour remains constant for one hour. When a colorimeter is used read at 540 $m\mu$.

Interferences. 1. If the solution is not shaken reproducibly after each addition the colour development is disturbed; 2. Sunlight affects the colour development; 3. Fe gives a marked reduction in colour — after the Norit treatment there is no further Fe in the extract; 4. Mn 30 ppm; 5. Ca 2000 ppm (turbidities); 6. Al 50 ppm.

2.1.5.8. *Determination of phosphate.* Principle: Molybdenum blue coloration with $SnCl_2$ as reducing agent.

Reagents. 1. Standard solution 10 ppm P: dissolve 0,439 g of KH_2PO_4 in Morgan extracting solution, make up to 1 litre; 2. Molybdenum reagent according to Peech: dissolve 15 g of $(NH_4)_6Mo_7O_{24}$. 4 aq in 300 ml of distilled water. While swirling add carefully 500 ml of HCl (s.g. 1,19). Make up to 1 litre with distilled water; 3. $SnCl_2$ 0,5 %: dissolve 0,5 g of $SnCl_2$ in 10 ml of HCl (s.g. 1,19), make up to 100 ml with distilled water. Prepare a fresh solution every day; 4. Mixed reagent: dissolve 1 part of molybdenum reagent according to Peech with 2 parts of Morgan extracting solution.

Procedure. To 2 ml of extract, diluted if necessary, add successively:

1. 3 ml of mixed reagent; within 10 minutes add;
2. 0,3 ml of $SnCl_2$ 0,5 %.

Measurement. After 10 minutes the colour remains sufficiently constant for 50 minutes. When a colorimeter is used read at 600 $m\mu$.

Interferences. 1. Fe 100 ppm; 2. Mn 10 ppm; 3. NO_3 40 ppm; 4. NO_2 5 ppm.

2.1.5.9. *Determination of nitrate with brucine.* Principle: In a concentrated sulphuric acid medium nitrates colour brucine yellowish-orange.

Reagents. 1. Standard solution 10 ppm NO_3: dissolve 0,137 g of $NaNO_3$ in Morgan extracting solution, dilute to 100 ml. Dilute this solution 1 : 99 with Morgan extracting solution; 2. brucine 2 % in Morgan extracting solution; 3. sulphuric acid A.R. (s.g. 1,84).

Procedure. To 2 ml of the extract, diluted if necessary, add sucessively:

1. 0,2 ml of brucine 2 %.
2. 3 ml of sulphuric acid. Keep shaking until the red colour has turned into a yellowish-orange one.

Measurement. After 30 minutes the colour remains constant for $2^1/_2$ hours. When a colorimeter is used read at 470 $m\mu$.

Interferences. 1. NO_2 5 ppm; 2. Fe 10 ppm. After the Norit treatment there is no further Fe in the extract; 3. Colourless organic compounds which cannot be removed with Norit (e.g. sugars) give a brown coloration; 4. Ca 3000 ppm.

2.1.5.10. *Determination of nitrate with pyrogallol.* Principle: In a concentrated sulphuric acid medium pyrogallol gives a violet coloration with nitrates.

Reagents. 1.Standard solution 10 ppm NO_3: dissolve 0,137 g of $NaNO_3$ in Morgan extracting solution, make up to 100 ml. Dilute this solution with Morgan extracting solution in 1 : 99 ratio; 2. Ferous solution 100 ppm: dissolve 0,125 g of $FeSO_4.7aq$ in Morgan extracting solution, make up to 250 ml; 3. Saccharose 0,1 % in distilled water; 4. Mixed solution: dissolve 12,5 g of hydroxylamine HCl, 50 ml of ferous solution, 7,5 ml of saccharose solution and 1 ml of 1000 ppm NO_3, make up to 250 ml with distilled water (can be kept for a week); 5. Pyrogallol solution: dissolve 6,25 g of pyrogallol and 0,75 g of $NaHSO_3$, make up to 250 ml with distilled water (can be kept for a week); 6. Sulphuric acid A.R. s.g. 1,84.

Procedure. To 2 ml of extract, diluted if necessary, add successively:

1. 1 ml of mixed solution and pyrogallol solution mixed in equal parts before addition. The mixed reagent turns blue and this coloration ultimately forms a precipitate (i.e. after about 1 hour). As long as the reagent is still clear it can be used. The hydroxylamine-HCl is used for preserving the ferrous salt in the mixed solution and for reducing ferric ions in the extract. The ferrous interference with the determination is constant when 5 to 25 ppm. Fe are present. The presence of ferrous ions in the mixed solution is used for reaching the threshold value of 5 ppm of Fe in all samples and the standard solution. The saccharose allows the standard solution to have a colour of its own comparable to the colourless organic compounds dissolved in the samples and not removed by the Norit. The $NaHSO_3$ in the pyrogallol solution serves to prevent oxidation of the pyrogallol.
2. 2 ml of sulphuric acid. The method of adding and mixing the reagents determine the colour formation. The best results are obtained by trying to keep two layers during the addition and then mixing by rapidly moving up and down the flattened end of a stirring rod in the test tube.

Measurement. After one hour the colour development is at its maximum for 30 minutes. When a colorimeter is used read at 595 mμ.

Interferences. 1. Nitrites are also determined. When 1 drop of urea 50 % and 0,2 ml of sulphuric acid (s.g. 1,84) are added before the determination the nitrite is removed in 10 minutes. The standard series should then be subjected to the same treatment; 2. Fe 25 ppm; 3. Cl 4000 ppm; 4. Ca 800 ppm (turbidities); 5. Exceptionally large amounts of dissolved organic material are not removed with Norit.

2.1.5.11. *Determination of ammonia.* Principle: Coloration with Nessler's reagent. Since in this case turbidity occurs in the Morgan extracting solution all extracts are diluted with distilled water in a 1 : 1 ratio. The standard series is also made up of Morgan extracting solution diluted 1 : 1 with distilled water.

Reagents. 1. Standard solution 20 ppm NH_4: dissolved 0,0732 g of $(NH_4)_2SO_4$ in Morgan extracting solution diluted 1 : 1 with distilled water. Make up to 1 litre; 2. Nessler's reagent (stock solution): dissolve 50 g of HgI_2 and 40 g of KI in about 100 ml of distilled water; dissolve 100 g of NaOH in about 300 ml of distilled water. After cooling mix the two reagents and make up to 500 ml; 3. Nessler's reagent (working solution): mix 1 part of Nessler's stock solution with 9 parts of distilled water (can be kept for a week); 4. Gum arabic solution: dissolve 10 g in about 150 ml of distilled water. Add 5 ml of Nessler's reagent (stock solution) and make up to 200 ml. Keep in the dark for 14 days, after which the supernatant opalescent liquid is siphoned off and stored in a brown flask; 5. Tartrate reagent: dissolve 80 g of

sodium tartrate and 20 g of NaOH, make up to 500 ml with distilled water. After cooling add 50 ml of gum arabic solution and make up to 1 litre.

Procedure. To 2 ml of extract, diluted if necessary, add successively (samples are diluted with distilled water in 1 : 1 ratio):

1. 3 ml of tartrate reagent. The tartrate reagent prevents Mg and Fe turbidity.
2. 7 ml of Nessler's working solution. If the extract is shaken immediately after the addition and fresh reagents are used little or no trouble from turbidity occurs.

Measurement. The measurements should be taken after 5 but within 20 minutes. Turbidity sometimes prevents the colorimetric determination.

Interferences. 1. Mn 5 ppm; 2. Mg 500 ppm; 3. Ca 5000 ppm; 4. Fe 20 ppm.

2.1.6. *Determination of reducible manganese*

For the purpose of comparison with data in the literature the Morgan extracting solution is in this case replaced by NH_4 acetate 1 N extract to which is added a reducing agent. The extraction ratio is also changed to 1 : 10. The analysis is practically identical to that described in the case of the manganese determination with formaldoxime (2.1.5.6.).

Reagents. 1. Extracting solution: dissolve 77,0 g of ammonium acetate and 2 g of hydroxylamine-HCl, make up to 1 litre. The solution can be kept for a week; 2. EDTA: 1 % in distilled water. For other reagents see 2.1.5.6.

Extraction. Shake mechanically for one hour 5 g of soil and 0,3 g of Norit SX-25 with 50 ml of extracting solution. Filter (Delta 314 3/4) and dilute 1 : 1 with distilled water since the undiluted medium interferes with the determination.

Procedure. See 2.1.5.6. Little $CaCO_3$ is dissolved from the soil by the extracting solution so that 1 ml of 1 % EDTA can be added immediately after the KCN solution.

Measurement. After half an hour the colour remains constant for $2^1/_2$ hours. When a colorimeter is used read at 450 mμ.

Interferences. See 2.1.5.6.

2.2. Plant samples

Na, K, Ca, Mg, P and Kjeldahl N are determined by the regional laboratories in a single sample obtained following digestion according to LINDNER and HARLEY.

Pretreatment of the samples. After the harvest the samples should be dried at 40°—80° C. The temperature should not exceed 80° C to avoid loss of nitrogen.

The results should be converted to material dried to 105° C. A sample should therefore be taken for a separate moisture determination at the same time as others are taken for the digestion.

Digestion of the sample according to Lindner and Harley. Weigh out 0,6 g of air dry plant material in a 100 ml graduated flask. Add 5 ml of sulphuric acid (s.g. 1,84). Experiments have shown that 1 to 2 ml of sulphuric acid are used of the amount originally added. For this reason only 4 ml of sulphuric acid are added in the blank determination. Heat on an electric plate.

The treated sample turns brownish-black. It should be swirled regularly to prevent foam formation. Should foam enter the neck of the flask not more than 1 to 2 drops of 30 % hydrogen peroxide should be added along the neck (as regards the hydrogen peroxide A.R. standard is suitable, since with other qualities phosphates and nitrogen

compounds are often made use of for stabilisation purposes). After the violent foam formation has subsided the mass is digested on the hotplate for a further 10 to 15 minutes. 5 to 10 drops of hydrogen peroxide are then added. If the reaction is too violent (the hotplate too hot) it is advisable to allow the flasks to cool before adding the hydrogen peroxide. 10 minutes later another 5 to 10 drops of hydrogen peroxide are added. This is repeated until the solution is clear. The hotplate is now raised to full heat (300° C). If the sample remains colourless after 10 minutes have elapsed the digestion is complete. Fill the graduated flask to 100 ml with distilled water.

2.2.1. *Determination of sodium, potassium and calcium*

Experiments have shown that when the Kipp flame photometer is used it makes little difference whether the comparison is made with a mixed standard containing Na, K and Ca in a 1 : 2 : 5 molar ratio or with separate standard series. Only in the case of Na are the results of the mixed standard approximately 3 % higher. Since both Na and Ca are found in the plant samples (usually in about the same ratio as in the mixed standard) it is preferred to use a mixed standard. The alternative for the Na determination is an extremely time consuming Ca separation which is unnecessary in view of the small analysis error in the mixed standard.

Reagents: 1. Stock solution: dissolve 3.003 g of $CaCO_3$, 0,894 g of KCl and 0,350 g of NaCl in a 600 ml beaker by adding sufficient HCl 1 N to dissolve the lot (about 60 ml). Then transfer quantitatively to a 1 litre graduated flask, make up with distilled water; 2. Standard solutions: pipette 100 ml of the stock solution into a 1 litre graduated flask, make up with distilled water to about 950 ml. Add 4 ml of sulphuric acid (s.g. 1,84) and bring up to volume with distilled water. This standard solution has a concentration corresponding to 100 mmol of Na, 200 mmol of K and 500 mmol of Ca/100 g of air dry plant material; 3. Standard series: pipette 0, 2, 5, 10, 20, 40, 60, 80 and 100 ml of the standard solution in 100 ml graduated flasks according to TABLE 2. Make up with dilute sulphuric acid (4 ml of sulphuric acid s.g. 1,84/litre). The standard series gives a direct reading as shown in TABLE 2; 4. $MgCl_2$ 5,00 N (standardise to chloride). The standard series can then be compared with each other when a fresh $MgCl_2$ solution is used.

TABLE 2.

ml of standard solution per 100 ml	millimoles per 100 g of airdry matter		
	Na	K	Ca
0	0	0	0
2	2	4	10
5	5	10	25
10	10	20	50
20	20	40	100
40	40	80	200
60	60	120	300
80	80	160	400
100	100	200	500

Procedure. Sodium and Potassium. Pipette 1 ml of the digested sample into test-tube and add 9 ml of distilled water. Compare with the standard series using a flame photometer.

Procedure. Calcium. Pipette 1 ml of the digested sample into test tubes and add 9 ml of distilled water. Then pipette 10 ml of the standard series into test-tubes. Add 2,5 ml of $MgCl_2$ 5,00 N to both standard series and diluted unknown solution. Mix and compare with the standard series using a flame photometer.
The testtubes employed should always be cleaned with nitric acid 4 N after each determination, and afterwards with tapwater and distilled water. Use a separate set of test-tubes for the flame photometer determination as they are very soon contaminated with sodium. For this purpose it is also advisable to use hardglass test tubes (Thermax, Leerdam, Holland).

2.2.2. *Determination of Kjeldahl nitrogen*

Principle: Nitrogen compounds are converted into ammonia by digesting samples in the manner described by Lindner and Harley. After distillation in an alkaline medium the ammonia is taken up in boric acid and titrated with potassium bi-iodate (SILVERSTEIN and PERTHEL, 1950).

Reagents. 1. NaOH 50 % in distilled water; 2. H_3BO_3 1 % in distilled water; 3. $KH(IO_3)_2$ 0,01 N: dissolve 3,899 g of $KH(IO_3)_2$ in distilled water, make up to 1 litre; 4. Mixed indicator: dissolve 0,15 g of bromocresol green and 0,1 g of methyl red in 200 ml of ethanol.

Procedure. Pipette 5 ml of the digested sample into a micro-distillation apparatus. Add about 2 ml of NaOH 50 % and distill over in 10 ml of boric acid 1 % to which are added 6 drops of mixed indicator. Then titrate to the colour of the blank with $KH(IO_3)_2$ 0,01 N.
If more than 10 ml of $KH(IO_3)_2$ are used in the titration it is advisable to repeat the distillation with a smaller amount of digested sample.

Calculation: $\frac{100}{3}$ ml of $KH(IO_3)_2$ 0,01 N = 1 mmol of N per 100 g of air dry plant material.

2.2.3. *Determination of phosphate*

Principle: Molybdenum blue coloration with the "Photorex" reducer.

Reagents. 1. Reduction mixture: dissolve 0,5 g of metol (p-methylaminophenolsulphate), 2,5 g of sodium sulphite and 75 g of sodium bisulphite in distilled water and make up to 1 litre; 2. Molybdenum solution: dissolve 25 g of $(NH_4)_6\ Mo_7O_{24}.4aq$ in 250 ml of H_2SO_4 10 N and make up to 1 litre with distilled water; 3. Sodium acetate solution: dissolve 340 g of $CH_3COONa.3aq$ in distilled water and make up to 1 litre; 4. Standard solution: dissolve 0,816 g of KH_2PO_4 in distilled water and make up to 1 litre. Dilute 1 : 9 with distilled water; 5. Standard series: pipette 0—0,1—0,2—0,3—0,4—0,5—0,6—0,7—0,8 and 0,9 ml of standard solution into 10 test tubes. Add 0,5 ml of the blank and make up to 2,5 ml with distilled water. This standard solution has a direct reading of 0, 2, 4, 6, 8, 10, 14, 16, and 18 mmol of PO_4/100 g of air dry matter.

Procedure. Successively pipette into test tubes 0,5 ml of sample and 2 ml of distilled water. Add 0,5 ml of reduction mixture and 0,5 ml of molybdenum solution to both the standard solution and the treatments. Let stand for 20 minutes then add 1 ml of sodium acetate solution.

Measurement. A reading can be taken 10 minutes later. When a colorimeter is used read at 600 mμ.

2.2.4. *Determination of magnesium*

Principle: Thyazol yellow is coloured red by $Mg(OH)_2$.

Reagents. 1. Dilute sulphuric acid: Add 40 ml of H_2SO_4 (s.g. 1,84) to 800 ml of distilled water and make up to 1 litre; 2. Mixed reagent: the Mg mixed reagent is the same as employed for the Mg determination in soil (see 2.1.5.7.); 3. KCN solution: dissolve 40 g of NaOH and 5 g of KCN in distilled water, dilute to 100 ml. Filter off the carbonates immediately prior to using; 4. Standard solution: dissolve 0,1478 g of $MgSO_4.7aq$ with dilute sulphuric acid, make up to 1 litre; 5. Standard series: pipette 0—0,2—0,4—0,8—1,2—1,6 and 2,0 ml of standard solution into test-tubes. Make up to 2,0 ml with dilute sulphuric acid. This standard series gives a direct reading of 0, 1, 2, 4, 6, 8 and 10 mmol of Mg/100 g of air dry plant material.

Procedure. Pipette into test tubes 2 ml of the sample: 1 ml for the replicate. Make up the replicates to 2 ml with dilute sulphuric acid. Add successively to the standard series and treatments 1,5 ml of mixed reagent and 1,0 ml of KCN solution. Shake vigorously and store in the dark for 1 hour.

Measurement. When a colorimeter is used read at 540 $m\mu$. If the replicates do not correspond or the concentrations are too high in the unknown solutions they should be diluted with dilute sulphuric acid.

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