Determination of fixed ammonium in soils

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DETERMINATION OF FIXED AMMONIUM IN SOILS

by

James Anthony Silva

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

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SECTION I. INTRODUCTION

Until recently, it was generally assumed that only a small proportion (<2%) of the total nitrogen in soils was in inorganic forms of combination. However, it is now well established that many soils have the capacity to fix ammonium (i.e., to adsorb ammonium in a manner such that it is not readily exchangeable) (Barshad, 1951; Allison et al., 1951, 1953b; Hanway and Scott, 1956; Nõmmik, 1957; Jansson, 1958; Stojanovic and Broadbent, 1960; Walsh and Murdock, 1960), and recent work has shown that some soils contain significant amounts of fixed (i.e., non-exchangeable) ammonium which is not determined by the methods normally used for estimation of inorganic forms of nitrogen in soils (Rodrigues, 1954; Dhariwal and Stevenson, 1958; Stevenson and Dhariwal, 1959; Bremner and Harada, 1959; Bremner, 1959; Walsh and Murdock, 1960; Schachtschabel, 1961a, b; Young, 1962; Young and Cattani, 1962). Present data indicate that the proportion of soil nitrogen as fixed ammonium usually does not exceed about 5% in surface soils, but that it can exceed 30% in certain subsoils. The mechanism of ammonium fixation by soils has not been fully elucidated, but it is known that both organic and inorganic soil constituents have the ability to fix ammonium, and it is currently assumed that the fixed ammonium in soils is in the lattices of silicate minerals (Nõmmik, 1957; Mortland, 1958; Jansson, 1958).

Several methods of determining fixed ammonium in soils have been proposed, but the methods adopted in most investigations concerning the occurrence and distribution of fixed ammonium in soils have been procedures involving the use of hydrofluoric acid (HF) for release of
ammonium held in soil minerals. The use of HF for determination of fixed ammonium in soils was originally proposed by Rodrigues (1954), but the HF treatment he adopted is very drastic, and there is evidence that it is subject to serious interference by soil organic matter (Bremner, 1959). Dhariwal and Stevenson (1958) and Bremner (1959) have proposed milder HF procedures for estimation of fixed ammonium, and these methods have been used in most investigations concerning fixed ammonium in soils. However, Schachtschabel (1961b) has recently proposed an HF method involving a pretreatment with H₂O₂ to reduce interference by soil organic matter, and he found that this method gave much higher values than the Bremner's method. This raises considerable doubts concerning the reliability of the methods currently available for determination of fixed ammonium in soils, and the value of research involving these methods cannot be assessed until these doubts are resolved.

Development of a reliable method for determination of ammonium fixed by inorganic soil constituents is essential for all investigations concerning ammonium fixation by the organic or inorganic fractions of soils. Fixation of ammonium by soil organic matter involves conversion of ammonium to organic forms of nitrogen. This means that to determine the amount of ammonium fixed by organic matter when soils are treated with ammonium, it is necessary to determine the total amount of ammonium fixed and to subtract the amount fixed by inorganic soil constituents.
SECTION II. LITERATURE REVIEW

The literature on ammonium fixation by soils and minerals has been reviewed by Reitemeier (1951), van der Marel (1954), Kardos (1955), and Mortland (1958). Therefore, only the portions of this literature which are pertinent to the present study will be discussed.

Definition of Fixed Ammonium

The Soil Science Society of America (1962) has proposed that ammonia fixation be defined as "adsorption of ammonium ions by soils or minerals in such form that they are neither water soluble nor readily exchangeable." However, it is well established that the extent to which ammonium and other cations fixed by soils and clay minerals are removed by cation-exchange methods depends very largely upon the nature of the cation used, and that the amount removed by another fixable cation (e.g., potassium, rubidium, cesium) is considerably less than the amount removed by a nonfixable cation (e.g., sodium, calcium). Also, several workers have shown that the release of fixed ammonium is blocked effectively by the presence of a small amount of K⁺ in the reagent used to effect release (e.g., Allison and Roller, 1955a; Hanway and Scott, 1956; Hanway et al., 1957; Nömmik, 1957). These findings, and proposals by Barshad (1951) concerning definition of ammonium fixation, have led to apparently general agreement that fixed ammonium be defined as adsorbed ammonium which is not replaceable by potassium. However, this definition is also inadequate, because the amount of ammonium released by K⁺ from soils and minerals containing fixed ammonium depends upon the type of K⁺ reagent used and the conditions of the treatment with this reagent.
(Scott et al., 1960). This means that, to define fixed ammonium, it is necessary to specify the method of determination even when $K^+$ is used as the replacing cation. In practice, most workers have found it convenient to define fixed ammonium in soils as ammonium which is not extractable by $\text{NH}_4\text{KCl}$ at laboratory temperatures; and, if this definition is adopted, it seems logical to define exchangeable ammonium as ammonium which is extractable by this reagent. These definitions have, therefore, been adopted in this dissertation. However, when various pretreatments are used to remove exchangeable ammonium and organic matter prior to HF treatments used for the determination of fixed ammonium, the implied definition of fixed ammonium is "the ammonium which is not removed by the pretreatment, but which is released by the HF solution." This is an arbitrary definition based on methodology, and is inherent in all HF methods involving a pretreatment for removal of exchangeable ammonium and organic matter. The research reported in this dissertation is limited to the fixed ammonium found in soil before addition of ammonium. This fixed ammonium has been described as "native" or "indigenous" fixed ammonium to differentiate it from ammonium fixed on addition of ammonium to soils and minerals. Neither of these terms is completely satisfactory because they have the questionable implication that this ammonium was fixed at the time of formation of the minerals in the soil. However, for lack of a better term, the fixed ammonium studied in the present work will be referred to as native fixed ammonium.

Fixed Ammonium in Soils

Rodrigues (1954) was the first to obtain evidence that soils
contained native fixed ammonium. He showed that a considerable fraction of the nitrogen in some tropical soils was not removed by customary cation-exchange methods of extraction, hydrogen peroxide oxidation, chromic-sulphuric acid oxidation at 100°C., or boiling 10% sulphuric acid, but was immediately released as ammonium by treatment with hydrofluoric acid. He concluded that this ammonium was derived from ammonium held by clay minerals and that 14 to 78% of the total nitrogen in the soils he examined was in the form of fixed ammonium. He also noted that the proportion of total nitrogen as fixed ammonium increased with depth in the profile and concluded that this was probably responsible for the pronounced decrease in C:N ratio with depth found in some of the profiles studied. Subsequent work has indicated that from 0.7 to 12.9% of the total nitrogen in surface soils and from 1.2 to 44% of the total nitrogen in subsoils is in the form of fixed ammonium. (Dhariwal and Stevenson, 1958; Stevenson et al., 1958; Bremner, 1959; Stevenson and Dhariwal, 1959; Gouny et al., 1960; Walsh and Murdock, 1960; Cheng, 1961; Young, 1962; Young and Cattani, 1962; Smirnov and Fruktova, 1963)

The quantity of native fixed ammonium in soils appears to be related to the amount and kind of clay mineral present (illite > montmorillonite > kaolinite) (Stevenson et al., 1958; Stevenson and Dhariwal, 1959)
The accepted view at present is that silicate minerals (montmorillonites, illites and vermiculites) are largely responsible for fixation of ammonium in soils (Reitemeier, 1951; Kardos, 1955; Mortland, 1958). Stevenson (1962) studied the nitrogen in rocks and minerals and found that 77 to 99% of the total nitrogen in metamorphic rocks (slate, gneiss, granite gneiss) and 66 to 99% of the total nitrogen in silicate minerals
(illite, muscovite, biotite, vermiculite, orthoclase feldspar) was present as fixed ammonium. This finding has been supported by the work of Adams (1962).

Fixation of Ammonium by Soils and Minerals

The ammonium-fixing capacity of a soil or mineral is usually defined as the amount of added ammonium retained by the soil or mineral against removal with K+ reagents. Much of the research which has been done on the ammonium-fixing capacities of soils, including the pioneer work of McBeth (1917), Chaminade and Drouineau (1936), and Chaminade (1940), has been reviewed by Reitemeier (1951) and Kardos (1955). Allison et al., (1951, 1953a, b) found that the quantity of ammonium fixed by soils from ammonium salt solutions varied with the conditions of fixation and the predominant mineral. Soils high in vermiculite were found to fix more ammonium under moist conditions than those containing large amounts of illite or montmorillonite. Illitic soils fix more ammonium when air-dried or oven-dried than when kept moist after addition of ammonium, whereas montmorillonitic soils fix little, if any, ammonium unless oven-dried after treatment with ammonium (Barshad, 1951; Harada and Kutsuna, 1954, 1955; Hanway and Scott, 1956; Leggett, 1958; Axley and Legg, 1960; Gouny et al., 1960; Smirnov and Fruktova, 1963). In studies of the ammonium-fixing capacities of several Wisconsin soils under moist, frozen, and oven-dry conditions, Walsh and Murdock (1960) found that frozen soils fixed nearly twice as much ammonium as did moist soils. Young and Cattani (1962) compared the fixation of anhydrous ammonia and aqua ammonia by air-dry soils and found good correlation between the amounts
of anhydrous ammonia and of aqua ammonia fixed by soil minerals. However, they found that the amount of anhydrous ammonia fixed by air-dry soils was much higher than the amount of aqua ammonia fixed by these soils. They deduced that ammonium from salts or aqua-ammonia must be carried by the soil solution to fixing sites, whereas anhydrous ammonia injected into air-dry soil can diffuse through the soil and reach fixing sites without first dissolving in the soil solution. They postulated that under dry field situations, fixation of ammonium from anhydrous ammonia is more rapid and extensive than fixation from other sources of ammonium. The ammonium-fixing capacities of rocks and minerals have been studied by Barshad (1954b), Allison and Roller (1955b) and Adams (1962). Adams (1962) found that all the minerals he studied were able to fix ammonium, but that their fixing capacities were much smaller than those of soils. He reported that the fixation of added ammonium by minerals decreased in the order: mica > feldspar > beryl > quartz.

It is now established that ammonium added to soil is fixed not only by the mineral fraction, but also by the organic fraction of soil. Ammonium in the form of anhydrous ammonia or aqua ammonia reacts with soil organic matter, leaf litter, and peat to form complex organic nitrogen compounds (Mattson and Koutler-Andersson, 1942, 1943; Harmsen and van Schreven, 1955; Mortland, 1958; Sohn and Pech, 1958; Burge and Broadbent, 1961; Nömmik and Nilsson, 1963). Mattson and Koutler-Andersson (1942,1943) found that ammonium fixation by organic matter is accompanied by a simultaneous consumption of oxygen and have postulated that this ammonium fixation is an autoxidative process involving aromatic compounds containing two or more phenolic hydroxyl groups and ammonium in converted
to organic forms of nitrogen. Several workers have reported that ammonium from sources other than anhydrous or aqua ammonia may also be fixed by organic matter. For example Broadbent et al. (1961) found that addition of neutral ammonium salts to soils led to some fixation of ammonium by organic matter, and Nõmmik and Nilsson (1963) found that ammonium released by decomposition of urea and calcium cyanamide in soils was also fixed by organic matter. Nõmmik and Nilsson (1963) further observed that the amount of ammonium fixed by organic matter was approximately doubled when the source of ammonium was calcium cyanamide instead of ammonium hydroxide. In agreement with other workers, they found that the extent of ammonium fixation by organic matter increased with increasing pH. Sohn and Pech (1958) deduced that fixation of ammonia from anhydrous ammonia by soils is likely to exceed the fixation of ammonium from neutral ammonium salts because ammonia can react with soil organic matter to form organic nitrogen compounds as well as become trapped in the lattices of minerals. They also concluded that at least 50% of the ammonia fixed by the surface layer of some New York mineral soils was fixed by soil organic matter. Also, McIntosh (1962) found that 50 to 90% of the total ammonium fixed by the surface mineral soils of Iowa treated with ammonium hydroxide was fixed by the organic fractions of these soils. Several workers (e.g., Burge and Broadbent, 1961; McIntosh, 1962; Nõmmik and Nilsson, 1963) have obtained significant positive correlations between the amounts of ammonium fixed by organic soils and the carbon contents of these soils.
Factors Affecting Fixation of Ammonium by Soils and Minerals

The factors which affect the fixation of ammonium by soils and minerals have been studied by many workers (e.g., McBeth, 1917; Joffe and Kolodny, 1937; Joffe and Levine, 1947a, b; Stanford and Pierre, 1947; Bower, 1950; Allison et al., 1951, 1953b; Reitemeier, 1951; Aomine and Wada, 1952a, c; Harada and Kutsuna, 1954; Nõmmik, 1957; Jansson, 1958; Leggett, 1958; Edwards, 1960; Stojanovic and Broadbent, 1960; Ghildyal and Singh, 1961; Rich, 1961; Schachtschabel, 1961b; Shoji and Matsumi, 1961; Kaila, 1962; McIntosh, 1962; Young and Cattani, 1962). Following is a brief summary of the results of these studies.

Amount and concentration of ammonium

The amount of ammonium fixed increases with increase in the amount and concentration of ammonium added (McBeth, 1917; Stanford and Pierre, 1947, McIntosh, 1962).

Time

Fixation of ammonium by soil is most rapid during the first few minutes but continues for several days (McBeth, 1917; Harada and Kutsuna, 1954; Nõmmik, 1957; Leggett, 1958; McIntosh, 1962). Several workers (e.g., Nõmmik, 1957; Leggett, 1958) have found ammonium fixation by clay minerals and soils to be 70 to 90% complete after the first hour of ammoniation.

pH

Ammonium fixation by soils, clay minerals, and soil organic matter increases with increase in pH (Nõmmik, 1957; Ghildyal and Singh, 1961, McIntosh, 1962).
Temperature and moisture

The ammonium-fixing capacity of soil is reduced or completely destroyed if soil is heated to 200°C. or higher temperatures before addition of ammonium (McBeth, 1917; Harada and Kutsuna, 1954). On the other hand, fixation of ammonium is more rapid at 30 to 70°C. than at room temperature (Aomine and Wada, 1952a; Harada and Kutsuna, 1954; Nõmmik, 1957). Air-drying after addition of ammonium increases the amount of ammonium fixed by vermiculite and illite and also promotes fixation by montmorillonite, which fixes little, if any, ammonium under moist conditions.

All three of these minerals show increased fixation when dried at 100°C. after addition of ammonium (Bower, 1950; Allison et al., 1951, 1953b; Harada and Kutsuna, 1954; Jansson, 1958; Stojanovic and Broadbent, 1960).

Cations

The ammonium-fixing capacity of soil saturated with various cations has been found to decrease in the order: Na⁺ > Mg²⁺ > Ca²⁺ > Ba²⁺ > Al³⁺ > H⁺ > Rb⁺ > K⁺ (McBeth, 1917; Stanford, 1948; Aomine and Wada, 1952c; Harada and Kutsuna, 1954; Nõmmik, 1957). Potassium causes the greatest reduction in ammonium-fixing capacity, and it has been found that the quantity of ammonium fixed by soils is inversely proportional to the amount of potassium applied before addition of ammonium. These findings, and the fact that the sum of the ammonium and potassium fixed is a constant, have been interpreted as indicating that these cations are fixed by the same mechanism (Stanford and Pierre, 1947). Nõmmik (1957) found that when ammonium and potassium were added to Swedish soils in equivalent amounts, they were fixed in a proportion of 3.3:1.
Anions

The ammonium-fixing capacity of soil appears to be unaffected by most anions, but several workers have reported that the fixation of ammonium added as phosphate is larger or smaller than that of ammonium added as sulfate or chloride (Harada and Kutsuna, 1954; Leggett, 1958).

Clay content

Some workers (Schachtschabel, 1961a; Kaila, 1962) have reported a close positive relationship between native fixed ammonium or ammonium-fixing capacity and clay content, but others (Ghildyal and Singh, 1961; Young and Cattani, 1962) have observed no relationship between clay content and native fixed ammonium or ammonium-fixing capacity.

Organic matter

Both ammonium and potassium fixation by the mineral fraction of soil have been found to be reduced by organic matter, presumably because organic matter reacts with minerals to block the sites involved in fixation (Joffe and Levine, 1947b; Ghildyal and Singh, 1961; Kaila, 1962).

Mechanism of Ammonium Fixation

Since the present work is concerned with ammonium fixation by the inorganic fraction of soil, only the literature concerning the mechanism of ammonium fixation by this fraction will be discussed here.

The silicate minerals which have been shown to fix ammonium are montmorillonite, illite, and vermiculite (Page and Baver, 1940; Joffe and Levine, 1947a; Stanford and Pierre, 1947; Barshad, 1948; Gieseking, 1949; Aomine, 1951; Allison et al., 1953a, b, c, 1955b; van der Marel, 1954). These minerals all have 2:1 (silica:alumina) lattices. Minerals
with 1:1 lattices have not shown appreciable fixation of ammonium and Joffe and Levine (1947a) found that kaolinite (a 1:1 lattice mineral) did not fix potassium or ammonium. Illite and vermiculite can fix ammonium under moist conditions but montmorillonite fixes ammonium only if it is dried or heated after addition of ammonium. Fixation by illite and vermiculite is increased by drying or heating.

A mechanism for the fixation of cations was suggested by Page and Baver (1940) as a result of their investigations with Wyoming bentonite and a colloid from Miami silt loam. They postulated that fixation occurs through trapping of cations in the cavities formed by the hexagonal oxygen rings of the silica sheets of two superimposed layers of a 2:1 type mineral. These cavities have a radius of approximately 1.35 Å, and cations such as K⁺, NH⁴⁺, Rb⁺, Cs⁺, and Ba²⁺, which have radii near this value, can be trapped within these cavities as the clay is dehydrated and the adjacent layers contract. The cations present in the cavities are in close proximity to the negative charges in the lattice and exert a strong attraction for the two adjacent clay plates, which results in contraction of the mineral lattice and a "locking" of the layers against rehydration and re-expansion. The cation thus trapped within the cavities has no possibility for exchange with other cations in the external solution, i.e., is "fixed." Its release must be preceded by expansion of the lattice. Page and Baver found that cations with radii larger than 1.35 Å were not fixed because they could not enter the cavities whereas cations with radii smaller than 1.35 Å were not fixed, because, although they could enter the cavities, they were too small to bind the two layers together. Barshad (1948, 1950, 1951), van der Marel (1954) and Leggett
(1958) have shown by x-ray diffraction studies that ammonium fixation causes the collapse of a 14 Å mineral to a 10 Å mineral, which lends support to the Page and Baver theory.

The forces of attraction between the negative charges in the clay lattices and the interlayer cations of silicate minerals were studied by Wear and White (1951). They found that the distances between the potassium ion and the negative charges in the tetrahedral and octahedral sheets were 2.19 Å and 4.99 Å, respectively. Since the force of attraction is inversely proportional to the square of the distance, they concluded that the forces associated with the charges in the tetrahedral sheets should be more than four times as great as the forces associated with the charges in the octahedral sheets, and that beidellite, illite, and vermiculite should fix more K⁺ than montmorillonite because they have relatively more charges arising in the tetrahedral sheets.

Page and Baver (1940) and Stanford and Pierre (1947) obtained evidence that potassium and ammonium are fixed in clay minerals by the same mechanism. Stanford and Pierre (1947) found a correlation coefficient of 0.87 between the moist fixation capacities of Webster high-lime soils for potassium and ammonium, and also observed that the sum of fixed potassium and fixed ammonium was nearly constant regardless of the order in which these two ions were added to these soils.

The location of the fixing sites in soil has been studied by determining the ammonium-fixing capacities and the native fixed ammonium contents of the sand, silt and clay fractions of soil (Barshad, 1951; Harada and Kutsuna, 1954; Nömmik, 1957; Gildyal and Singh, 1961; Schachtschabel, 1961b; Kaila, 1962; Young and Cattani, 1962; van Schreven,
1963). Some workers have obtained results indicating that the clay fraction is largely responsible for ammonium fixation (Schachtschabel, 1961b; Kaila, 1962), but others have found that the silt fraction is responsible for as much, if not more, ammonium fixation than the clay fraction (Barshad, 1951; Harada and Kutsuna, 1954; Nömmik, 1957). However, the methods used for dispersion of soil and separation of the particle size fractions in these studies are open to serious criticism.

Methods of Determining Ammonium-Fixing Capacity

The procedure generally used to determine the ammonium-fixing capacity of a soil or mineral is to saturate a sample of the material under analysis with an ammonium solution and subsequently, extract the material with a salt solution and determine the ammonium content of the extract. The ammonium fixed by the material is calculated from the difference between the ammonium added and that recovered by the extracting solution. Extraction is usually performed with solutions of KCl (Bower, 1950; Aomine, 1951; Aomine and Wada, 1952c; Harada and Kutsuna, 1954) but other extractants have been used. Stojanovic and Broadbent (1960) found that KCl- HCl solutions (pH 1.0) and NaAc- HAc solutions (pH 4.8) gave similar result with soils, but Scott et al. (1958) found that the amount of ammonium extracted from ammonium-saturated vermiculite by N KCl or N NaCl was increased by acidification of these reagents. Allison et al. (1953b) found that CaCl₂ solution recovered more ammonium from ammonium-saturated soils than did KCl solution, and concluded that this was due to the ability of K⁺ to contract the lattices of silicate minerals and prevent release of ammonium. Most workers have not performed extractions of
control samples to allow for exchangeable ammonium present in the unsaturated material, presumably because they assumed that failure to allow for this ammonium would not cause significant error in the value for ammonium-fixing capacity. Nõmmik (1957) recognized that this was a potential source of error, and he proposed a method in which KCl solution is used for extraction and a control analysis is performed.

A modification of the extraction method involves determination of the ammonium retained by the soil after extraction, rather than of the ammonium in the extract. In this modification, the total nitrogen contents of extracted ammonium-saturated and extracted unsaturated samples are determined by Kjeldahl analysis, and ammonium-fixing capacity is calculated from the difference in the results of these analyses. This procedure was adopted by Allison et al. (1951) who used KCl solution for extraction and by Allison et al. (1953b) who used KCl and CaCl₂ solutions as extractants. Kaila (1962) also employed CaCl₂ solution for extraction, but used a method involving digestion with concentrated sulfuric acid in the absence of Kjeldahl digestion catalysts for determination of the ammonium not removed by CaCl₂ solution. Sohn and Peech (1958) used 10% NaCl for extraction, but they determined both exchangeable ammonium and total nitrogen in ammonium-saturated and unsaturated samples, and calculated ammonium-fixing capacity by subtracting (total-minus exchangeable NH₄⁻) N in the control from (total-minus exchangeable NH₄⁻) N in the ammonium-saturated sample. They considered that this method was superior to the procedure used by Allison et al. (1953b).

Barshad (1951) studied the release of ammonium from ammonium-saturated soils by distillation with NaOH, Ba(OH)₂, MgO, and KOH, and concluded that
the simplest method of determining the ammonium-fixing capacity of soil was to distill an ammonium-saturated sample with NaOH and a duplicate ammonium-saturated sample with KOH, and to calculate ammonium-fixing capacity from the difference in the amounts of ammonium released by these distillations. This duplicate distillation procedure is based on the assumption that NaOH releases all added ammonium (exchangeable and fixed), and that KOH releases only exchangeable ammonium. However, it has been found that small quantities of K⁺ block the release of fixed ammonium from soils and minerals by distillation with NaOH (Allison and Roller, 1955a; Hanway and Scott, 1956; Hanway et al., 1957; Nömmik, 1957; Dhariwal and Stevenson, 1958). Hanway et al. (1957) found that no fixed ammonium was released from vermiculite by distillation with NaOH solutions containing more than 2 K⁺ ions per 100 Na⁺ ions. Another assumption made in the duplicate distillation procedure is that the amount of ammonium liberated from organic nitrogen compounds during distillation of soil with KOH solution is identical to the amount released by distillation with NaOH solution of equivalent concentration. This assumption may be valid if the distillations are conducted under exactly the same conditions, but in practice it is very difficult to control the rate of distillation, and the amount of ammonium liberated from soil organic matter by alkaline distillation depends upon the rate as well as the time of distillation (Bremner, 1959). Allison and Roller (1955a) found that the duplicate distillation procedure of estimating ammonium-fixing capacity gave lower values than their extraction-Kjeldahl digestion method with soils which had been heated after ammonium saturation and higher values with soils that were not heated after saturation. They concluded that the latter
result was due to fixation of exchangeable ammonium during distillation with KOH. Allison and Roller (1955b) found that the duplicate distillation method gave lower results with ammonium-saturated minerals that had been leached with various salt solutions before analysis. Nömmik (1957) obtained similar values for the ammonium-fixing capacity of soil by the KCl extraction-Kjeldahl digestion method, the duplicate distillation method, and Rodrigues' (1954) HF method.

Leggett (1958) and Leggett and Moodie (1962) have proposed a method in which the soil sample is treated with a known amount of ammonium as N\_NH\textsubscript{4}OAc and ammonium fixing capacity is calculated by determining the ammonium liberated by aeration of this sample with K\textsubscript{2}CO\textsubscript{3} for 16 hours, and then subtracting the ammonium liberated by similar aeration of an unsaturated sample. This method involves the assumption that aeration of soil with K\textsubscript{2}CO\textsubscript{3} for 16 hours does not lead to release of significant amounts of ammonium from soil organic nitrogen compounds. Leggett and Moodie (1962) found that this aeration method had greater precision than the extraction-Kjeldahl digestion procedure proposed by Allison et al. (1951), but gave similar values for ammonium-fixing capacity with most soils.

The ammonium-fixing capacities obtained by all of the methods discussed above are those of the organic and inorganic fractions of soil combined and this fact must be considered in interpretation of data obtained by these methods.
Methods of Determining Native Fixed Ammonium

Two types of methods have been used for determination of native fixed ammonium in soils. In one, the soil sample is distilled with NaOH, and a duplicate sample is distilled with KOH, and fixed ammonium is estimated from the difference between the amounts of ammonium released by the two distillations. In the other, the soil sample is treated with hydrofluoric acid to decompose the minerals containing the fixed ammonium, and the ammonium released by this treatment is determined. Exchangeable ammonium is removed before the treatment with hydrofluoric acid (Dhariwal and Stevenson, 1958; Bremner, 1959; Schachtschabel, 1961a, b) or is estimated separately and subtracted from the ammonium released by hydrofluoric acid (Rodrigues, 1954; Bremner, 1959).

The duplicate distillation procedure was originally proposed by Barshad (1951) as a method for estimation of the ammonium-fixing capacity of soil, but Hanway and Scott (1956) and Leggett (1958) have used it for determination of native fixed ammonium in soils. They found that this method gave much lower values than the HF method of Rodrigues (1954). Leggett (1958) also used a variation of the duplicate distillation method in which native fixed ammonium was estimated from the difference in the amounts of ammonium liberated by aeration of duplicate samples of soil with Na$_2$CO$_3$ and K$_2$CO$_3$ solutions of equivalent concentration, and found that the results by this technique were similar to those obtained by the Barshad's procedure. The value of these distillation and aeration methods for determination of native fixed ammonium in soils seems very dubious, because it has been shown that small amounts of K$^+$ effectively block the release of fixed ammonium by NaOH or Na$_2$CO$_3$ solution.
Moreover, these methods involve the questionable assumption that the amount of ammonium released from organic nitrogen compounds by treatment of soil with KOH or K$_2$CO$_3$ solution is identical to the amount released by similar treatment with NaOH or Na$_2$CO$_3$ solution of equivalent concentration.

The methods currently favored for determination of native fixed ammonium in soils are essentially modifications of the HF method proposed by Rodrigues (1954). In Rodrigues' method, the soil sample is treated with a mixture of 4 volumes of 40% HF and 1 volume of 50% sulfuric acid for 1 hour at room temperature, and the ammonium released by this treatment is estimated by distilling the extract with alkali after removal of HF by heating with sulfuric acid. This is a rather drastic procedure, and there is evidence that it causes extensive decomposition of organic nitrogen compounds to ammonium (Bremner, 1959). Dhariwal and Stevenson (1958) and Bremner (1959) proposed milder HF procedures for estimation of native fixed ammonium, and their methods have been adopted in most investigations concerning the occurrence and distribution of nonexchangeable ammonium in soils.

In the method of Dhariwal and Stevenson, the soil sample is heated with N KOH in an autoclave for 8 hours at 20 pounds pressure and a temperature of about 120°C. to remove exchangeable ammonium and decompose labile organic nitrogen compounds, and the residue from this treatment is washed with 0.5 N KCl and treated with 5 N:HF:0.75 N HCl:0.6 N H$_2$SO$_4$ at room temperature for 12 to 16 hours with occasional shaking. The entire
sample is then distilled with NaOH, and fixed ammonium is calculated from
the amount of ammonium liberated by this distillation.

In the method proposed by Bremner, the soil sample is shaken with
\( N \text{ HF: } N \text{ HCl} \) at room temperature for 24 hours, and the ammonium released
by this treatment is estimated by filtering the soil-acid mixture and
distilling an aliquot of the neutralized filtrate with borate buffer
(pH 8.8). Exchangeable ammonium in the sample is estimated separately
and subtracted from the ammonium released by the HF-HCl treatment to
obtain the fixed ammonium value. This method was based on studies which
indicated that the relatively mild treatments used for release and esti­
mation of fixed ammonium in this procedure should not cause significant
release of ammonium from organic nitrogen compounds. However, evidence
was obtained that ammonium release from soil organic matter may sometimes
be significant, and the inclusion of a pretreatment involving a short
period of heating with 0.5 \( N \) KOH was recommended to minimize interference
by organic nitrogen compounds.

To determine native fixed ammonium in studies of Wisconsin soils,
Walsh and Murdock (1960) adopted a method involving the pretreatment
recommended by Dhariwal and Stevenson (1958), the HF treatment used by
Bremner (1959), and estimation of the ammonium released by distillation
of the soil-HF mixture with MgO.

Schachtschabel (1961a, b) recently proposed a method of determining
native fixed ammonium which involves estimation of the ammonium released
by HF and \( H_2SO_4 \) after treatment of the soil sample with hydrogen peroxide
to destroy organic matter, and subsequent correction of this value for
ammonium released during the HF treatment from organic nitrogen compounds.
not removed by the peroxide treatment. In this method, the soil sample is heated with H\textsubscript{2}O\textsubscript{2} (to oxidize organic matter) in the presence of KCl (to prevent fixation by soil minerals of ammonium produced by oxidation of organic nitrogen compounds), and the residue from this treatment is washed with 0.05 N HCl, water and ethanol, and dried and weighed. A subsample of the dried material is then heated with concentrated H\textsubscript{2}SO\textsubscript{4} and 40% HF to decompose the soil minerals, and the amount of ammonium liberated by this treatment is determined by distillation with NaOH. Fixed ammonium is calculated from this ammonium value by subtracting 3 mg. of ammonium-N for every 100 mg. of carbon in the subsample of peroxide-treated soil used for the treatment with HF (to correct for ammonium released from organic compounds during this treatment).

Schachtschabel (1961a) found that this method of estimating native fixed ammonium in soils gave much higher values than Bremner's HF-HCl procedure with the 0.5 N KOH pretreatment omitted, and he concluded that the latter method gave low results. However, he presented no evidence to support this conclusion, and his use of a standard correction for ammonium released from organic matter is open to criticism, because his data showed that the calculated correction varied between 2 and 4 mg. of ammonium-N per 100 mg. of carbon in the peroxide-treated soil.

The use of a pretreatment to remove organic matter in HF methods of estimating native fixed ammonium in soils is theoretically desirable, because removal of organic matter should reduce the risk of interference by organic nitrogen compounds and render the minerals containing fixed ammonium more susceptible to attack by HF. However, it is essential that the pretreatment should not cause significant release of fixed ammonium.
or lead to fixation of ammonium. The possibility that some of the ammonium liberated from soil organic matter during pretreatment of soils with hot KOH solution is fixed by minerals appears very remote (Hanway and Scott, 1956; Dhariwal and Stevenson, 1958). However, Stevenson (1962) recently observed that prolonged treatment with hot N KOH leads to release of fixed ammonium from igneous rocks and primary minerals, which suggests that the pretreatment with N KOH used in the Dhariwal and Stevenson method of determining native fixed ammonium may cause release of fixed ammonium from soil minerals.

It is clear from this brief review that considerable doubts exist concerning the reliability of the methods currently available for determination of fixed ammonium in soils, and that the value of research involving these methods cannot be assessed until these doubts are resolved. Development of a reliable method of determining ammonium fixed by inorganic soil constituents is essential for all investigations concerning ammonium fixation by the organic or inorganic fractions of soils, because fixation of ammonium by soil organic matter involves conversion of ammonium to organic forms of nitrogen, and, to determine the amount of ammonium fixed by organic matter when soils are treated with ammonium, it is necessary to determine the total amount of ammonium fixed and to subtract the amount fixed by inorganic soil constituents. It may eventually become possible to develop a direct method of determining ammonium fixation by soil organic matter, but this possibility appears very remote.
SECTION III. MATERIALS AND METHODS

Soils

The soils used (Table 1) were selected to obtain a wide range in organic matter content and to include samples used in previous studies of ammonium fixation. Before use, the samples were air-dried and ground to pass a 100-mesh sieve.

Samples 1, 2 and 6 were from permanent grass plots on the Iowa State University Farm, and sample 3 was from the check plot (no. 934) in the five-year CCOMM rotation experiment on this farm. Samples 4 and 5 were from the Organic Matter Maintenance Experiment (continuous corn plots) at the Soil Conservation Experimental Farm, Shenandoah, Iowa. Sample 4 was collected from plots (C-8) receiving 8 tons of corn stalks per acre annually, and sample 5 was from plots (A-8) receiving annual additions of 8 tons of alfalfa per acre. Sample 7 was from plot no. 205 of the rotation-fertility experiment on the Pasture Experimental Farm, Albia, Iowa. The rotation was CCOM and the plot was sampled during first year corn. Sample 8 was a Texas soil referred to in work by Thompson et al. (1954) as sample 776C. Samples 9 and 10 were supplied by F. E. Allison, U. S. Department of Agriculture. Sample 9 was from the B2 horizon of a Clarence silt loam clay in Ford County, Illinois, and sample 10 was from the subsoil of a Yolo clay loam near Winters, California. A sample of peat from the Colo Bog, Iowa, was also used for some studies. Before use, this sample was treated 3 times with N\textsubscript{H}F:N\textsubscript{H}Cl (40 ml./g. of peat) to remove mineral material. Each treatment was performed by shaking the peat-acid mixture for 24 hours, and the residue from the
Table 1. Analyses of soils

<table>
<thead>
<tr>
<th>No.</th>
<th>Soil Type</th>
<th>Depth</th>
<th>pH</th>
<th>Organic C</th>
<th>Total N</th>
<th>Ammonium-N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Clay minerals&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Particle size distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>inch.</td>
<td></td>
<td>%</td>
<td>ppm.</td>
<td>E</td>
<td>F</td>
<td>Sand</td>
</tr>
<tr>
<td>1</td>
<td>Glencoe sicl</td>
<td>0-6</td>
<td>6.7</td>
<td>10.2</td>
<td>120</td>
<td>29</td>
<td>158</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>Webster cl</td>
<td>0-6</td>
<td>6.9</td>
<td>5.22</td>
<td>270</td>
<td>25</td>
<td>124</td>
<td>MIK</td>
</tr>
<tr>
<td>3</td>
<td>Webster cl</td>
<td>0-6</td>
<td>6.7</td>
<td>3.18</td>
<td>2590</td>
<td>7</td>
<td>108</td>
<td>MIK</td>
</tr>
<tr>
<td>4</td>
<td>Marshall sicl</td>
<td>0-6</td>
<td>4.9</td>
<td>2.20</td>
<td>2090</td>
<td>23</td>
<td>268</td>
<td>MIK</td>
</tr>
<tr>
<td>5</td>
<td>Marshall sicl</td>
<td>0-6</td>
<td>5.5</td>
<td>2.14</td>
<td>1970</td>
<td>10</td>
<td>265</td>
<td>MIK</td>
</tr>
<tr>
<td>6</td>
<td>Nicollet sacl</td>
<td>0-6</td>
<td>7.0</td>
<td>1.56</td>
<td>1340</td>
<td>7</td>
<td>85</td>
<td>MIK</td>
</tr>
<tr>
<td>7</td>
<td>Marion sil</td>
<td>0-6</td>
<td>6.2</td>
<td>1.27</td>
<td>1240</td>
<td>16</td>
<td>182</td>
<td>MIK</td>
</tr>
<tr>
<td>8</td>
<td>Austin sicl</td>
<td>0-6</td>
<td>8.1</td>
<td>1.09</td>
<td>840</td>
<td>1</td>
<td>92</td>
<td>---</td>
</tr>
<tr>
<td>9</td>
<td>Clarence sicl</td>
<td>15-23</td>
<td>6.0</td>
<td>0.87</td>
<td>922</td>
<td>24</td>
<td>217</td>
<td>IMVK</td>
</tr>
<tr>
<td>10</td>
<td>Yolo cl</td>
<td>15-23</td>
<td>7.6</td>
<td>0.79</td>
<td>813</td>
<td>15</td>
<td>273</td>
<td>MIK</td>
</tr>
</tbody>
</table>

<sup>a</sup>E, exchangeable; F, nonexchangeable (fixed).

<sup>b</sup>Sicl, silty clay loam; cl, clay loam; sacl, sandy clay loam; sil, silt loam; sic, silty clay.

<sup>c</sup>M, montmorillonite; I, illite; V, vermiculite; K, kaolinite. Predominant mineral is listed first.
treatments was washed thoroughly with water and dried under vacuum over concentrated sulfuric acid.

Five of the soils selected had been used in previous work on ammonium fixation in soils. Samples 1, 2, and 7 were used by McIntosh (1962), and samples 9 and 10 by Allison et al. (1953a, b), Hanway (1954), and Hanway and Scott (1956).

Clay minerals

**Vermiculite**

The sample of vermiculite used was prepared from a bulk sample of unexfoliated 'Veri-Gro' ore obtained by J. M. Bremner from Dupré Vermiculite (Exfoliators) Ltd., Welwyn, England. Before use, it was broken down to <300-mesh size by sonic vibration in an M. S. E. Ultrasonic Disintegrator (60-watt, 18-20 kc.) fitted with a 3/4-inch titanium probe. This was accomplished by subjecting 25 g. of vermiculite (<40 mesh) suspended in 75 ml. of water to sonic vibration for 10 minutes, the suspension being stirred continuously during this treatment. The suspension was then transferred to a 300-mesh sieve and the material remaining on the sieve was washed with deionized water and subjected to further sonic treatment. This procedure was continued until the entire sample had passed the 300-mesh sieve. The <300-mesh material was separated by filtration in an auto-irrigator and dried under vacuum over concentrated sulfuric acid. The dried material was ground in agate mortar and stored in a tightly-stoppered bottle.

**Ammonium-saturated vermiculite**

This was prepared by shaking 40 g. of the <300-mesh vermiculite with
400 ml. of $\text{NH}_4\text{Cl}$ for 18 hours. The mixture was filtered with suction through a sintered-glass funnel, and the residue on the filter was leached with an additional 400 ml. of $\text{NH}_4\text{Cl}$ and washed with 95% ethanol until the leachate gave a negative test for chloride with $\text{AgNO}_3$. The ammonium-saturated vermiculite was then dried under vacuum over concentrated sulfuric acid and ground in an agate mortar to pass a 100-mesh sieve.

Analytical Procedures

**pH**

This was determined by a Beckman Zeromatic glass-electrode pH meter, a soil:water (1:2.5, w/v) suspension being used for determination of soil pH.

**Total nitrogen**

This was determined by the semimicro Kjeldahl procedure of Bremner (1960) modified to include the pretreatment with water recommended by Bal (1925) for Kjeldahl analysis of clay soils. In this modified method, a sample of soil or clay mineral containing about 1 mg. of nitrogen was treated with 1 ml. of water in a 30-ml. micro-Kjeldahl digestion flask and the mixture was shaken and allowed to stand for about 15 minutes. Concentrated sulfuric acid (3 ml.) and $\text{K}_2\text{SO}_4: \text{CuSO}_4: \text{Se}$ mixture (1.1 g.) were then added and the mixture was digested on an electric micro-Kjeldahl digestion rack for 5 hours after the digest became clear. After cooling, the digest was steam-distilled with $\text{NaOH}$, and the ammonia liberated was collected in boric acid-indicator solution and determined by titration with 0.01 N $\text{H}_2\text{SO}_4$. 
Exchangeable ammonium

This was determined by the extraction-distillation or direct distillation method described by Bremner (1964). In the extraction-distillation method, a sample of soil or mineral was shaken for 1 hour with 2 N KCl (10 ml./g. of soil or 0.2 g. of mineral). The mixture was then filtered and an aliquot of the extract was steam-distilled with 0.2 g. of ignited, heavy MgO for 3 to 4 minutes in the apparatus described in Section VII (Figure 2). The ammonia liberated by distillation was collected in 5 ml. of boric acid indicator solution and determined by titration of the distillate with 0.005 N H$_2$SO$_4$. In the direct distillation method, a 2-g. sample of soil or 0.2-g. sample of mineral was placed in a 100-ml. distillation flask and treated with 10 ml. of 2 N KCl and 0.1 g. of ignited MgO. Exchangeable ammonium was determined by collection and titration of the ammonia liberated by steam distillation of this mixture for 3 to 4 minutes as described in Section VII.

Nonexchangeable (fixed) ammonium

The analyses for fixed ammonium-N reported in Table I were performed by the KOBr-HF method (procedure A) described in Section VII.

Organic carbon

Two methods were used for determination of organic carbon in soils. A modification of the method of Mebius (1960) was used for most of the analyses reported. In this method, a sample of soil containing about 5 mg. of organic carbon was treated with 5 ml. of water, 5 ml. of N K$_2$Cr$_2$O$_7$ and 15 ml. of concentrated H$_2$SO$_4$, and the mixture was boiled under reflux for 30 minutes and cooled. Residual K$_2$Cr$_2$O$_7$ was then determined by titration with 0.2 N (NH$_4$)$_2$SO$_4$·FeSO$_4$·6H$_2$O (Mohr's salt) using
N-phenylanthranilic acid as indicator. This method is subject to interference by halides, and it was found to give erroneously high values when used for determination of organic carbon in soil samples subjected to treatments with solutions containing chloride or bromide even when the samples were washed thoroughly with $\text{N}_2\text{K}_2\text{SO}_4$ and water after these treatments. Organic carbon in these samples was therefore determined by a modification of the method of Allison (1960), which is not subject to interference by halides. In this modification of the Allison method, a sample of soil containing about 15 mg. of organic carbon was boiled for 10 minutes with 3 g. of $\text{K}_2\text{Cr}_2\text{O}_7$, 3 ml. of water and 25 ml. of a 3:2 (v/v) mixture of concentrated $\text{H}_2\text{SO}_4$ and 85% $\text{H}_3\text{PO}_4$. The $\text{CO}_2$ evolved was conducted by a stream of $\text{CO}_2$-free air through a series of traps containing a 50% solution of KI, a saturated solution of $\text{Ag}_2\text{SO}_4$, granular zinc, and anhydrous magnesium perchlorate. The purified $\text{CO}_2$ was collected in a weighed Nesbitt absorption bulb containing "Mikohbite" and anhydrous magnesium perchlorate and determined from the increase in weight of this bulb.

**Particle size analysis**

This was performed by the pipette method of Kilmer and Alexander (1949) as modified by Edwards and Bremner (1964). Dispersion was effected by shaking a 10-g. sample of soil with 1.5 g. (dry weight) of a sodium-saturated cation-exchange resin (Amberlite IRC-50) and 180 ml. of water for 10 hours.

**Identification of clay minerals**

The clay minerals in the soils used were identified by X-ray diffraction analysis of clay and fine silt fractions separated by sedimentation
of soil samples previously treated with hydrogen peroxide to remove organic matter. X-ray analyses were performed on samples of these fractions which had been oriented on glass or porcelain slides and subsequently air-dried, air-dried and solvated with ethylene glycol, or air-dried and heated at 600°C. for 1 hour. Samples of untreated air-dried soils were also analyzed on a ring mount and after solvation with ethylene glycol on a bakelite mount. Analyses were performed using a General Electric XRD-5 diffractometer with filtered copper Kα radiation.¹

**Distillation and determination of ammonium**

Unless otherwise specified, distillation of ammonium was performed with steam using the distillation apparatus described in Section VII (rate of distillation, ca. 7.5 ml./min.), and the ammonia liberated by distillation was collected in boric acid-indicator solution and determined by titration with standard sulfuric acid as described in the same section.

**Miscellaneous**

**Reagents**

Unless otherwise stated, the chemicals used were reagent-grade quality and all water employed was distilled water (condensed steam) which had been deionized by treatment with ion-exchange resins in a Bantam Demineralizer (Model BD-1).

**Shaking and centrifuging**

In experiments requiring use of a mechanical shaker, a Precision

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¹The assistance of C. L. Ho and T. Demirel of the Engineering Experiment Station Laboratory, Ames, Iowa, in the performance and interpretation of these analyses is gratefully acknowledged.
Equipoise Heavy Duty Shaker (260 strokes per minute) was employed. In studies involving centrifugation, an International Centrifuge (Size 2) allowing centrifugation of 8 x 100-ml. centrifuge tubes at 2000 rpm. (ca. 1000 x g) was used.

**Moisture**

Unless otherwise stated, all analyses reported are on a moisture-free basis, moisture being estimated from loss in weight following drying at 110°C. for 12 to 18 hours.
SECTION IV. COMPARISON OF CURRENT HF METHODS FOR DETERMINATION OF FIXED AMMONIUM

Description of Methods

Five methods of determining fixed ammonium in soils involving the use of HF are presently described in the literature [Rodrigues, 1954; Dhariwal and Stevenson, 1958; Bremner (direct method), 1959; Bremner (KOH method), 1959; Schachtschabel, 1961b]. Brief descriptions of these methods are given in Table 2. Three of the methods include a pretreatment for removal of organic matter before HF treatment. The pretreatment used in the Dhariwal and Stevenson method involves autoclaving with N KOH at 120°C and 20 pounds pressure for 8 hours, and the pretreatment used in Bremner's KOH method involves boiling with 0.5 N KOH for approximately 30 minutes. The Schachtschabel method employs a pretreatment with 30% H₂O₂ and 1.3 N KCl. The HF treatments used for release of fixed ammonium range from the relatively mild treatment with N HF:N HCl used by Bremner to the rather drastic treatments with 40% HF and 50% or concentrated H₂SO₄ used by Rodrigues and Schachtschabel. The procedures also differ in the method and period of treatment with HF solution and in the technique used for estimation of the ammonium released by HF.

The objective in the work reported in this section was to compare the five methods described. Detailed descriptions of the procedures adopted in this comparison are given in Appendix I. An attempt was made to follow the original methods in every detail, but in some cases this was not possible owing to the lack of adequate information in the publications describing these methods.
Table 2. Methods for determination of fixed ammonium in soils involving use of hydrofluoric acid

<table>
<thead>
<tr>
<th>Method</th>
<th>Pretreatment to remove organic matter</th>
<th>Release of fixed ammonium</th>
<th>Distillation of ammonium released</th>
<th>Correction^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodrigues (1954)</td>
<td>----</td>
<td>40% HF and 50% H₂SO₄</td>
<td>NaOH</td>
<td>E</td>
</tr>
<tr>
<td>Dhariwal &amp; Stevenson (1958)</td>
<td>N KOH</td>
<td>5 N HF:0.75 N HCl:0.6 N H₂SO₄</td>
<td>NaOH</td>
<td>---</td>
</tr>
<tr>
<td>Bremner (direct) (1959)</td>
<td>----</td>
<td>N HF:N HCl</td>
<td>Sodium borate buffer (pH 8.8)</td>
<td>E</td>
</tr>
<tr>
<td>Bremner (KOH) (1959)</td>
<td>0.5 N KOH</td>
<td>N HF:N HCl</td>
<td>Sodium borate buffer (pH 8.8)</td>
<td>---</td>
</tr>
<tr>
<td>Schachtschabel (1961b)</td>
<td>30% H₂O₂ and 1.3 N KCl</td>
<td>40% HF and conc. H₂SO₄</td>
<td>NaOH</td>
<td>R</td>
</tr>
</tbody>
</table>

^aE, fixed ammonium is calculated by subtracting exchangeable ammonium; R, fixed ammonium is calculated by subtracting 3 mg. of ammonium-N for every 100 mg. of carbon in the peroxide-treated soil.

^bRodrigues did not specify the concentration of HF employed. It has been assumed that 40% HF was used.
Results and Discussion

The results obtained by analysis of 6 soils using the 5 methods described are presented in Table 3. It can be seen that the methods gave widely divergent results, and that the average fixed ammonium-N values for the 6 soils by the various methods decreased in the order: Rodrigues method, 917 ppm.; Schachtschabel method, 316 ppm.; Bremner direct method, 181 ppm.; Bremner KOH method, 170 ppm.; Dhariwal and Stevenson method, 166 ppm.

Table 3. Comparison of current HF methods for determination of fixed ammonium in soils

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Method</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>9</th>
<th>10</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rodrigues</td>
<td>1460</td>
<td>1260</td>
<td>1150</td>
<td>735</td>
<td>554</td>
<td>341</td>
<td>917</td>
</tr>
<tr>
<td></td>
<td>Dhariwal &amp; Stevenson</td>
<td>134</td>
<td>111</td>
<td>231</td>
<td>148</td>
<td>160</td>
<td>212</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Bremner (direct)</td>
<td>153</td>
<td>126</td>
<td>240</td>
<td>158</td>
<td>181</td>
<td>227</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>Bremner (KOH)</td>
<td>127</td>
<td>107</td>
<td>227</td>
<td>148</td>
<td>180</td>
<td>228</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>Schachtschabel</td>
<td>375</td>
<td>257</td>
<td>372</td>
<td>238</td>
<td>302</td>
<td>354</td>
<td>316</td>
</tr>
</tbody>
</table>

The method of Rodrigues gave much higher values than any of the other methods used, and the finding that the values by this method increased with increase in the organic matter content of the soil analyzed supports previous evidence (Bremner, 1959) that this method is subject to serious interference by organic soil nitrogen compounds.
The values by the Schachtschabel method are considerably higher than those obtained by the Dhariwal and Stevenson method or by Bremner's two methods, and the data in Table 3 confirm Schachtschabel's (1961a) finding that the values by his method are about twice those obtained by Bremner's direct method. It is difficult to account for this finding, because Schachtschabel's method involves both a pretreatment with H$_2$O$_2$ and KCl to reduce interference by organic nitrogen compounds and a correction to allow for ammonium derived from organic nitrogen compounds not removed by this pretreatment. It seems reasonable, therefore, to expect that Bremner's direct method, which involves no pretreatment or correction to minimize interference by soil organic matter, would give the higher values. However, the data in Table 3 show that there are relatively small differences between the results obtained by Bremner's two methods, which indicates that his direct method is not subject to extensive interference by organic nitrogen compounds. Alternative explanations for the large discrepancy between the results obtained by the Schachtschabel and Bremner methods are that the HF treatment used in Bremner's method does not effect complete release of fixed ammonium or that the pretreatment used in Schachtschabel's method leads to fixation by soil minerals of some of the ammonium produced by oxidation of soil organic matter during this pretreatment.

The values obtained by the Dhariwal and Stevenson method and by Bremner's two methods are not greatly different. The Dhariwal and Stevenson method gave a lower value than Bremner's direct method with each of the soils analyzed. When compared with Bremner's KOH method, it gave slightly higher values with soils 1, 2, and 4, the same value
with soil 6, and lower values with soils 9 and 10. Comparison of the results obtained by Bremner's two methods shows that the KOH method gave lower values with the soils containing more than 1% organic carbon (soils 1, 2, 4, 7), but nearly identical values with the soils containing less than 1% organic carbon (soils 9 and 10), and that the difference in the results by these methods decreased with decrease in the total-N content of the soil analyzed. This lends support to Bremner's (1959) conclusion that his direct method may be subject to some interference by organic nitrogen compounds and that this interference can be reduced by use of a pretreatment with 0.5 N KOH.

The possible sources of error in the HF methods compared may be summarized as follows:

1. The pretreatment used to eliminate interference by organic matter.

   This may release fixed ammonium or fail to remove organic nitrogen compounds that release ammonium under the conditions used for subsequent release and estimation of fixed ammonium. Also, it may lead to fixation by soil minerals of ammonium released from soil organic matter.

2. The HF treatment used for release of fixed ammonium.

   This may not effect complete release of fixed ammonium and it may lead to formation of ammonium from organic nitrogen compounds.

3. The method used to determine the ammonium released by HF treatment.

   This may not give quantitative recovery of ammonium and it may release ammonium from organic nitrogen compounds.

   These sources of error involved in the determination of fixed ammonium in soils will be investigated in the following sections.
SECTION V. EVALUATION OF PRETREATMENTS

Introduction

The objectives in evaluating pretreatments are to study the efficiencies of these pretreatments in removal of organic nitrogen compounds which could interfere in the subsequent determination of fixed ammonium, and to investigate the possible sources of error in these pretreatments.

The three pretreatments used for removal of organic nitrogen compounds in the current HF procedures are \( \text{H}_2\text{O}_2 - \text{KCl}, \text{N KOH}, \) and \( 0.5 \text{ N KOH}. \) The literature on the use of \( \text{H}_2\text{O}_2 \) for oxidation of soil organic matter indicates that the amount of soil organic matter destroyed by \( \text{H}_2\text{O}_2 \) varies from 33 to 98%, depending on the amount of organic matter in the soil and the method of \( \text{H}_2\text{O}_2 \) treatment used (Hosking, 1932; Waksman, 1938; Verdade, 1954; Turc, 1957). Alexander and Byers (1932) found that 73 to 90% of the organic carbon in several soils was removed by treatment with \( \text{H}_2\text{O}_2 \), and they also found that in one soil, 79% of the organic carbon and 79% of the total nitrogen was removed by this treatment. They reported that some of the nitrogen in the \( \text{H}_2\text{O}_2 \) residue of this soil was in the form of exchangeable ammonium-N. McLean (1931) found that repeated treatment of soils with \( \text{H}_2\text{O}_2 \) led to removal of from 93 to 97% of the total carbon and from 49 to 87% of the total nitrogen. Savage and Stevenson (1961) reported that about 80% of the carbon in soil humic acids was oxidized to \( \text{CO}_2 \) while the remaining carbon was oxidized to low molecular weight organic acids by \( \text{H}_2\text{O}_2 \) treatment at room temperature. They found nitrogen present in the inorganic form, as ammonia and nitrate, and also in the organic form, as amino acids, following \( \text{H}_2\text{O}_2 \).
treatment. Oxidation of soil organic matter has been performed under acid, neutral and alkaline conditions, but the most complete oxidation has been obtained under acid conditions. Under alkaline conditions, MnO₂ and CaCO₃ cause catalytic decomposition of H₂O₂ and little, if any, oxidation of organic matter occurs (Best, 1931; Alexander and Byers, 1932; Hosking, 1932; Waksman, 1938; Singh, 1954). These workers reported that treatment with acid prior to H₂O₂ treatment reduces interference from these sources almost completely. The foregoing discussion indicates that the H₂O₂ treatment does not effect complete removal of organic nitrogen from soil.

Pretreatments with KOH are considerably milder than pretreatments with H₂O₂ and differ in that removal of organic matter by KOH depends on extraction and hydrolysis rather than oxidation. Tinsley and Salam (1961) found that 69% of the nitrogen in soil was extracted by boiling for 1 hour with 0.5 N KOH whereas only 33% was extracted by prolonged shaking with 0.5 N KOH at room temperature. These results and the fact that Bremner (1954) reported that 20 to 40% of the soil organic matter is resistant to alkali extraction indicate that the KOH pretreatments do not effect complete removal of organic nitrogen from soil.

Since the investigations discussed above showed that complete removal of organic nitrogen from soil is not effected by treatment with either H₂O₂ or KOH, it appears very likely that the determination of fixed ammonium in soils by HF treatment following these pretreatments would be subject to interference by organic nitrogen compounds remaining in the pretreatment residues. Therefore, other methods for removing organic nitrogen were considered. Some of the characteristics of the pretreat-
ment desired were that it remove all the labile organic nitrogen and exchangeable ammonium without causing the release of fixed ammonium or allowing the fixation of ammonium from organic nitrogen compounds during the pretreatment and, that it be relatively simple and fast.

The search for the pretreatment desired was concentrated on the methods used for organic carbon determinations in soil since complete removal of carbon in the soil, which is the objective of these methods, should also result in removal of much, if not all, of the organic nitrogen in the soil. Dry combustion methods such as that described by Winters and Smith (1929) are considered the standard methods for determining carbon in soils and are considered the most accurate methods for this determination. The soil is heated to about 900°C to remove carbon in dry combustion methods, and this should also be very effective in removing organic nitrogen. However, Scott et al. (1956) found that fixed ammonium in ammonium-saturated illite was lost gradually at temperatures up to 200°C, and this loss continued with increasing temperature until complete removal of fixed ammonium occurred at 400°C. They also reported that ammonium-saturated vermiculite showed no loss of fixed ammonium until the temperature rose above 500°C. Rodrigues (1954) reported that soils treated with H₂O₂, and subsequently subjected to various temperatures lost fixed ammonium when the temperature rose above 450 to 500°C. Thus it appears that use of dry combustion methods for removing organic nitrogen can cause the loss of fixed ammonium from some soil minerals, especially at high temperatures. Therefore, they would be unsatisfactory for use prior to analysis for fixed ammonium.

Wet combustion methods in which soil is heated with an oxidizing
mixture of $K_2Cr_2O_7$, concentrated $H_2SO_4$ and $H_3PO_4$ for the determination of carbon have been described by many workers, including Walkley and Black (1934), van Slyke (1954), Shaw (1959), Allison (1960), Bremner and Jenkinson (1960), and Mebius (1960). However, strong acids used in these methods can destroy the clay minerals and release fixed ammonium, so these methods would not be suitable for removal of organic nitrogen compounds from soil prior to analysis for fixed ammonium.

In order to avoid the defect mentioned above for methods employing acid reagents, consideration was given to some alkaline oxidizing agents. Potassium permanganate has been used for oxidation and determination of organic matter in soils (Waksman, 1938; Wright and Schnitzer, 1959), but it did not effect complete removal of organic matter and thus gave variable results (Waksman, 1938). Therefore, it did not offer much promise of success in the present search. Alkaline persulfate is an oxidizing agent that has been used for oxidation of organic compounds in solutions and for oxidation of organic matter in soils. Robinson (1922) used ammonium persulfate to remove organic matter in mechanical analysis of soils, but found that during the treatment, sulfuric acid was formed and he reported that this acid liberated about 6.5% of the mineral material in one soil. Potassium persulfate has been used for the determination of carbon in aqueous solutions of organic compounds (Osburn and Werkman, 1932; Gertner and Ivekovic, 1954; Katz et al., 1954). Osburn and Werkman found that the persulfate solution became acid at the end of the reaction period, whereas Gertner and Ivekovic, and Katz et al. acidified the solution with $H_2SO_4$ prior to the determination. All three studies reported 94 to 100% recovery of added carbon
by this method. A modification of the potassium persulfate method which utilized N KOH to maintain an alkaline system was tested by heating a sample of soil under reflux with N KOH and potassium persulfate for several hours. Although the solution was alkaline at the beginning of the treatment, the pH of the supernatant at the end of the heating period was about 1.3. Therefore, no further trials were made with this reagent.

The hypobromites have been used as oxidizing agents for many years. Robinson (1922) used sodium hypobromite for removal of organic matter from soil prior to mechanical analysis and found that it was not too effective. Walkley (1935) compared several methods for determining organic carbon in soil and reported that treatment with sodium hypobromite at room temperature recovered only 65% of the carbon determined by dry combustion. On the other hand, Troell (1931) found that alkaline sodium hypobromite solution was very effective for removal of organic matter prior to mechanical analysis of soils, and recent work by Protz (1960), in which soil was heated with alkaline sodium hypobromite solution, showed that this procedure was very effective in removal of soil organic matter prior to mechanical analysis. The hypobromites have the ability to convert ammonium to nitrogen gas and this property has been utilized in the gasometric determination of urea (Stehle, 1921; van Slyke, 1926) and in the conversion of ammonium to nitrogen gas for analysis in the mass spectrometer (Rittenburg, 1948). It has been reported that the conversion of ammonium to nitrogen gas by hypobromite is not quantitative, but that 1.5 to 3.0% of nitrous oxide is produced during the reaction of sodium hypobromite with ammonium (Clusius and
Rechnitz, 1953) and that some of the ammonium is oxidized to nitrate during the reaction of ammonium with potassium hypobromite (Clusius and Bühler, 1954). This defect can be serious in quantitative analyses, but it is of little consequence in the removal of organic nitrogen from soil and the nitrate formed can be removed by washing the residue after hypobromite treatment. The hypobromites appeared to offer considerable promise for the removal of organic nitrogen from soils prior to determination of fixed ammonium. The use of potassium hypobromite would prevent the release of ammonium in fixed positions during the pretreatment and the ammonium formed on oxidation of soil organic matter by this reagent would be immediately converted to N₂ by the following reaction:

\[ 2\text{NH}_3 + 3\text{KOB}_r = \text{N}_2 + 3\text{KBr} + 3\text{H}_2\text{O}. \]

The rapid removal of ammonium-N released from organic compounds by this reaction as well as the high concentration of K⁺ in the solution should preclude the fixation by the soil minerals of ammonium released from organic matter during this pretreatment. The alkaline reaction of this reagent should minimize the danger of release of fixed ammonium by destruction of clay minerals.

Therefore, it was the objective of the studies reported in this section to develop a pretreatment procedure for the removal of organic nitrogen from soils using KOBr, and to evaluate this procedure in comparison with other pretreatments used in current HF methods for determining fixed ammonium.
Development of KOBr Pretreatment

Experiments were conducted to determine the optimum conditions for pretreatment of soil with KOBr solution. Potassium hypobromite tends to decompose to form potassium bromide and oxygen \((2\text{KOBr} = 2 \text{KBr} + \text{O}_2)\), but its rate of decomposition is reduced if it is kept at low temperature and is made strongly alkaline. It was decided, therefore, to use a freshly-prepared solution of KOBr which was prepared at low temperature and made approximately \(N\) with respect to KOH.

**Effect of period of pretreatment with KOBr solution**

**Preparation of KOBr solution** The solution used was prepared by adding 6 ml. of bromine dropwise (ca. 0.5 ml./min.) to 200 ml. of 2 N KOH, the solution being cooled (ca. 4°C.) in an icebath and stirred continuously during this addition.

**Pretreatment** One-gram samples of soils 1 and 5 were treated with 20 ml. of KOBr solution for 0.5, 2.0, 6.0 and 10.0 hours and the soil-KOBr mixtures were subsequently diluted with water and boiled for 5 minutes as described in Appendix II. The pretreatment residues were then separated and washed with 0.5 N KCl as described in Appendix II.

**Analyses of pretreatment residues** After washing with 0.5 N KCl, each pretreatment residue was shaken with 40 ml. of N HF:N HCl containing 1 drop of octyl alcohol for 24 hours at room temperature, and the ammonium released by this treatment was determined by the KOH distillation procedure described in Appendix III, the soil-acid mixture being transferred to a distillation flask with 20 ml. of water and subsequently distilled with 10 ml. of 10 N KOH. To allow for ammonium-N liberated from organic nitrogen compounds in the pretreatment residue.
under the conditions of the KOH distillation method, a duplicate sample of residue was treated with a mixture of 40 ml. of _N_ HF: _N_ HCl, 10 ml. of _N_ KOH and 20 ml. of water, and the ammonium-N released by steam distillation of this soil-ca. _0.3_ N KOH mixture as described in Appendix III was subtracted from the ammonium-N released by distillation with KC: after the treatment with HF-HCl solution.

**Results and discussion** The results presented in Table 4 show that more ammonium-N was released by HF treatment from samples which had been pretreated with KOBr for only 0.5 hours than from those which had been treated for 2 or more hours whereas increasing the period of KOBr treatment from 2 to 10 hours did not result in the release of markedly different amounts of ammonium-N. The complete removal of alkali-labile organic nitrogen compounds by KOBr pretreatment is indicated by the fact that no ammonium-N was released from pretreatment residues by distillation with KOH when the period of KOBr treatment was 2 to 10 hours. However, when the period of pretreatment was only 0.5 hours, 2 to 7 ppm. of ammonium-N were released by KOH distillation, which shows that complete removal of alkali-labile nitrogen compounds had not been effected in the short period of treatment. Therefore, it was decided to adopt the practice of treating the soil with KOBr solution at room temperature for 2 hours prior to boiling in all subsequent studies.
Table 4. Effect of period of pretreatment with KOBr solution on amount of ammonium-N released by shaking pretreated soil with $\text{NHF: }\text{NHC}_1$ (40 ml./g. of soil) for 24 hours

<table>
<thead>
<tr>
<th>Period of pretreatment (hr.)$^b$</th>
<th>Ammonium-N released (ppm. of soil)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil 1</td>
</tr>
<tr>
<td>0.5</td>
<td>149 (7)</td>
</tr>
<tr>
<td>2.0</td>
<td>142 (0)</td>
</tr>
<tr>
<td>6.0</td>
<td>141 (0)</td>
</tr>
<tr>
<td>10.0</td>
<td>142 (0)</td>
</tr>
</tbody>
</table>

$^a$Figures in parentheses are amounts of ammonium-N released by distillation of pretreated soils with ca. 0.3 N KOH.

$^b$Soil-KOBr mixture was boiled vigorously for 5 minutes after standing at room temperature for period indicated.

Effect of boiling in KOBr pretreatment

Methods One-gram samples of soils 1, 2, 5, and 9 were treated with 20 ml. of KOBr for 2 hours and boiled for 5 minutes as described in Appendix II. A sample of soil which was also treated with KOBr solution for 2 hours, but was not boiled, was transferred to a 100-ml. polypropylene centrifuge tube with 0.5 N KCl, centrifuged, and the supernatant decanted, then the sample was washed as described in Appendix II. The samples which had been boiled were also washed by the same procedure. Treatment of the KOBr-pretreatment residues with HF and KOH was performed as described in the preceding experiment.

Results and discussion The results (Table 5) show that more ammonium-N was released by HF treatment from samples which were unboiled (B) than from samples which had been boiled for 5 minutes (A).
difference in the ammonium-N released from the boiled and unboiled samples decreased with decreasing total nitrogen content of the soils (see Table 1). Boiling the soil-KOBr mixture resulted in more complete removal of alkali-labile organic nitrogen compounds so that no ammonium-N was released by KOH distillation of boiled samples, but varying amounts of ammonium-N were released by KOH distillation of unboiled samples. It was therefore decided to adopt the pretreatment involving boiling for 5 minutes after treatment of the samples with KOBr for 2 hours at room temperature.

Table 5. Effect of boiling in KOBr pretreatment on amount of ammonium-N released by shaking pretreated soil with N HF:N HCl (40 ml./g. of soil) for 24 hours

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Pretreatment(^a)</th>
<th>Ammonium-N released (ppm. of soil)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>141 (0)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>173 (30)</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>117 (0)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>136 (18)</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>242 (0)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>258 (14)</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>184 (0)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>190 (7)</td>
</tr>
</tbody>
</table>

\(^a\)A, soil was treated with KOBr solution at room temperature for 2 hours and mixture was subsequently boiled vigorously for 5 minutes; B, as in A, but mixture was not boiled.

\(^b\)Figures in parentheses are amounts of ammonium-N released by distillation of pretreated soils with ca. 0.3 N KOH.
Efficiency of Pretreatments

Comparison of H₂O₂-KCl, KOH and KOBr pretreatments

The experiments described below were designed to compare the efficiency of the H₂O₂-KCl pretreatment recommended by Schachtschabel (1961b), the N KOH pretreatment recommended by Dhariwal and Stevenson (1958), and the KOBr pretreatment developed in the present study. The pretreatments were applied to three soils (2, 7 and 9), and efficiency was evaluated by determining the amounts of organic carbon and nitrogen removed by each pretreatment.

H₂O₂-KCl pretreatment

A 10-g. sample of soil was treated with H₂O₂ and KCl as described in Appendix I, and the residue was dried at 50°C., weighed, and ground to pass a 100-mesh sieve.

N KOH pretreatment

A 50-g. sample of soil was autoclaved with 1,250 ml. of N KOH as described in Appendix I. The residue was washed 3 times with 500 ml. of 0.5 N KCl by decantation and subsequently transferred with 0.5 N KCl to a sintered glass funnel fitted with a Whatman no. 50 filter paper and washed thoroughly with 0.5 N KCl and water. The washed residue was then dried over concentrated H₂SO₄ under vacuum at room temperature, weighed, and ground to pass a 100-mesh sieve.

KOBr pretreatment

A 50-g. sample of soil was treated with 1000 ml. of KOBr as described in Appendix II. The residue was then washed and dried as in the pretreatment with N KOH and subsequently weighed and ground to pass a 100-mesh sieve.

Analyses of pretreatment residues

The pretreatment residues were analyzed for organic carbon, total nitrogen, exchangeable ammonium-N, and fixed ammonium-N. Organic carbon, total nitrogen, and exchangeable
ammonium-N were determined as described in Section III. Fixed ammonium-N was determined by a method in which the equivalent of 1 g. of untreated soil was shaken in a polypropylene centrifuge tube with 20 ml. of 5 N HF:N HCl at room temperature for 24 hours, and the ammonium released by this treatment was determined by the KOH distillation method described in Appendix III, the soil-acid mixture being transferred to the distillation flask with 20 ml. of water and subsequently distilled with 15 ml. of 10 N KOH. (The treatment with 5 N HF:N HCl was adopted because work reported in Section VI indicated that it effected complete release of fixed ammonium). To allow for ammonium-N liberated from organic nitrogen compounds in the pretreatment residues under the conditions of the KOH distillation method, the equivalent of 1 g. of untreated soil was treated with a mixture of 20 ml. of 5 N HF:N HCl, 15 ml. of 10 N KOH, and 40 ml. of water, and the ammonium-N released by steam distillation of this soil-ca. 0.3 N KOH mixture as described in Appendix III was subtracted from the ammonium-N released by distillation with KOH after the treatment with HF-HCl solution. Organic nitrogen in the untreated soils was calculated from total N minus (exchangeable ammonium plus fixed ammonium) -N, fixed ammonium-N being determined by the KOBr-HF method described in Section VII. The total nitrogen determination by the Kjeldahl method used in this study does not include much, if any, of the nitrate present in soil, so it was not considered in the calculation of organic nitrogen in the treated or untreated samples.

**Results and discussion** The results (Table 6) show that the efficiency of the three pretreatments in removing organic carbon and nitrogen decreased in the order: KOBr > H$_2$O$_2$-KCl > N KOH. Their average
Table 6. Amounts of organic carbon and nitrogen removed by treatment of soils with H$_2$O$_2$-KCl, N KOH, and KOBr

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil No.</th>
<th>Organic carbon</th>
<th>Total nitrogen</th>
<th>Ammonium-N$^a$</th>
<th>Organic nitrogen</th>
<th>% Removed by treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>2</td>
<td>52200</td>
<td>4270</td>
<td>25</td>
<td>124</td>
<td>4121</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>12700</td>
<td>1240</td>
<td>16</td>
<td>182</td>
<td>1042</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>8700</td>
<td>922</td>
<td>24</td>
<td>217</td>
<td>681</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>24530</td>
<td>2140</td>
<td>22</td>
<td>174</td>
<td>1948</td>
</tr>
<tr>
<td>H$_2$O$_2$-KCl</td>
<td>2</td>
<td>1800</td>
<td>451</td>
<td>32</td>
<td>180</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>900</td>
<td>301</td>
<td>12</td>
<td>190</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>900</td>
<td>409</td>
<td>16</td>
<td>240</td>
<td>153</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>1200</td>
<td>387</td>
<td>20</td>
<td>203</td>
<td>164</td>
</tr>
<tr>
<td>N KOH</td>
<td>2</td>
<td>6500</td>
<td>616</td>
<td>0</td>
<td>128</td>
<td>488</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2800</td>
<td>306</td>
<td>0</td>
<td>180</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1900</td>
<td>350</td>
<td>0</td>
<td>218</td>
<td>132</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>3730</td>
<td>424</td>
<td>0</td>
<td>175</td>
<td>249</td>
</tr>
<tr>
<td>KOBr</td>
<td>2</td>
<td>1600</td>
<td>180</td>
<td>0</td>
<td>124</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>500</td>
<td>182</td>
<td>0</td>
<td>182</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>700</td>
<td>245</td>
<td>0</td>
<td>217</td>
<td>28</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>870</td>
<td>202</td>
<td>0</td>
<td>174</td>
<td>28</td>
</tr>
</tbody>
</table>

$^a$E, exchangeable; F, fixed.
efficiencies in removing organic nitrogen were 98, 87, and 86%, respectively, and their average efficiencies in removing organic carbon were 96, 93, and 81%, respectively. It is noteworthy that KOBr was more efficient in removing organic nitrogen than organic carbon, whereas H$_2$O$_2$ was more efficient in removing organic carbon than organic nitrogen.

Organic nitrogen and carbon were removed to about the same extent by the KOH pretreatment.

The finding that the average fixed ammonium-N content of the H$_2$O$_2$-pretreated soils (203 ppm.) was considerably higher than the corresponding contents of the KOBr and KOH pretreated soils (174 and 175 ppm., respectively) has several possible explanations. One is that the KOBr and KOH pretreatments cause release of fixed ammonium. Another is that the H$_2$O$_2$-KCI pretreatment is less effective than the KOBr or KOH pretreatments in removal of organic nitrogen compounds that decompose with formation of ammonium when treated with HF-HCl solution. A third possible explanation is that the H$_2$O$_2$-KCl pretreatment leads to fixation by soil minerals of ammonium released by oxidation of soil organic matter during this pretreatment.

It is noteworthy that, in contrast to the KOBr and KOH pretreatment residues, the H$_2$O$_2$-KCl pretreatment residues contained appreciable amounts (12 to 32 ppm.) of exchangeable ammonium-N. This means that the technique used by Schachtschabel to wash the H$_2$O$_2$-KCl pretreatment residue is inefficient and leads to an erroneously high value in subsequent analysis of the residue for fixed ammonium.

**Efficiency of KOBr pretreatment**

Since the data reported in Table 6 indicated that the KOBr
pretreatment was more efficient than the H\textsubscript{2}O\textsubscript{2}-KCl and KOH pretreatments, it was decided to study the efficiency of this pretreatment with soils having a wide range in organic matter content. The seven soils selected for this study had organic carbon contents ranging from 10.2 to 0.9\% and organic nitrogen contents ranging from 0.9 to 0.07\%. Ten-gram samples of the soils were treated with 200 ml. of KOBr solution and the residues were washed with 0.5 \textit{N} KCl as described in Appendix II. The residues were then washed thoroughly with water, dried under vacuum over concentrated H\textsubscript{2}SO\textsubscript{4}, weighed, and ground to pass a 100-mesh sieve. Analysis of the residues were performed as in the work reported in Table 6.

The results (Table 7) show that the KOBr pretreatment was highly efficient in removal of organic nitrogen (and exchangeable ammonium) with all the soils tested, and that, on the average, it removed 93\% of the organic carbon and 98\% of the organic nitrogen in these soils.

Fixation of Ammonium during Pretreatments

During the pretreatment of soils for removal of organic nitrogen in the HF methods of determining fixed ammonium, one problem which may be encountered is that ammonium released from organic matter by pretreatment can be fixed by soil minerals. If fixation of ammonium occurs during pretreatment, serious error will result in the determination of fixed ammonium in soils. An experiment was designed to study fixation of ammonium from organic and inorganic sources by soil minerals during various pretreatments. Materials used in this experiment included vermiculite (<300 mesh) as the mineral, HF-treated peat as the source of organic nitrogen, and (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} as the source of inorganic nitrogen.
Table 7. Amounts of organic carbon and nitrogen removed by treatment of soils with KOBr

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Organic carbon</th>
<th>Total nitrogen</th>
<th>Ammonium-N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Organic nitrogen</th>
<th>% Removed by KOBr treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated soil</td>
<td></td>
<td></td>
<td>KOBr-treated soil</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organic carbon</td>
<td>Total nitrogen</td>
<td>Ammonium-N&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Organic nitrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ppm.</td>
<td>ppm. of untreated soil</td>
<td>ppm.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>102000</td>
<td>9120</td>
<td>29</td>
<td>158</td>
<td>8933</td>
</tr>
<tr>
<td>2</td>
<td>52200</td>
<td>4270</td>
<td>25</td>
<td>124</td>
<td>4121</td>
</tr>
<tr>
<td>3</td>
<td>31800</td>
<td>2590</td>
<td>7</td>
<td>108</td>
<td>2475</td>
</tr>
<tr>
<td>4</td>
<td>22000</td>
<td>2090</td>
<td>23</td>
<td>268</td>
<td>1799</td>
</tr>
<tr>
<td>6</td>
<td>15600</td>
<td>1340</td>
<td>7</td>
<td>85</td>
<td>1248</td>
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<tr>
<td>7</td>
<td>12700</td>
<td>1240</td>
<td>16</td>
<td>182</td>
<td>1042</td>
</tr>
<tr>
<td>9</td>
<td>8700</td>
<td>922</td>
<td>24</td>
<td>217</td>
<td>681</td>
</tr>
<tr>
<td>Average</td>
<td>35000</td>
<td>3080</td>
<td>19</td>
<td>163</td>
<td>2900</td>
</tr>
</tbody>
</table>

<sup>a</sup>E, exchangeable; F, fixed.
The pretreatments studied were:

1. No pretreatment
2. Extraction with 2 N KCl
3. Autoclaving with N KOH (according to Dhariwal and Stevenson, 1958)
4. Boiling with 0.5 N KOH (according to Bremner (KOH method), 1959)
5. Treatment with H_2O_2 and KCl (according to Schachtschabel, 1961b)
6. Treatment with KOBr

Methods

Each pretreatment was applied separately to: (1) 0.25 g. of vermiculite, (2) 0.25 g. of HF-peat, (3) 0.25 g. of vermiculite + 0.25 g. of HF-peat, and (4) 0.25 g. of vermiculite + 3 ml. of (NH_4)_2SO_4 solution containing 2.5 mg. of N. In pretreatment of vermiculite-ammonium sulfate mixture, the pretreatment solutions were added to vermiculite immediately before addition of ammonium sulfate. The pretreatments were performed as described in the Appendix II. For determination of fixed ammonium in vermiculite after pretreatment, the pretreatment residue was shaken with 20 ml. of N HF:N HCl for 24 hours at room temperature and the ammonium released by the HF treatment was estimated by the KOH steam distillation procedure as described in Appendix III. A separate sample of the pretreatment residue was treated with a mixture of 5 ml. of 10 N KOH, 20 ml. of N HF:N HCl, and 10 ml. of water and the ammonium-N released by steam distillation of the vermiculite-ca. 0.3 N KOH mixture as described in Appendix III was subtracted from the ammonium-N released by distillation with KOH after the treatment with HF-HCl solution to obtain the
value for fixed ammonium-N.

Results and discussion

In this experiment, vermiculite was allowed to come in contact with a large amount of ammonium from an organic or an inorganic source during pretreatment. The ammonium fixed by vermiculite during pretreatment was calculated as the difference between the amount of ammonium released by HF from the vermiculite + HF-peat mixture and the amounts of ammonium released from vermiculite and HF-peat individually, or the difference between the amount of ammonium released by HF from vermiculite + (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} and that released from vermiculite alone. Calculation of the amount of ammonium-N fixed by vermiculite during pretreatment with H\textsubscript{2}O\textsubscript{2} and KCl is illustrated in Table 8.

Table 8. Calculation of amount of ammonium-N fixed by vermiculite during pretreatment of vermiculite-peat and vermiculite-ammonium sulfate mixtures with H\textsubscript{2}O\textsubscript{2} and KCl (1.3 me. of KCl/g. of vermiculite)

<table>
<thead>
<tr>
<th>Mixture\textsuperscript{a}</th>
<th>Fixed ammonium-N in pretreatment residue</th>
<th>Ammonium-N fixed during pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>V  P  A</td>
<td>ug.</td>
<td>ug.  ug./g. of V</td>
</tr>
<tr>
<td>g.  g.  mg. N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 0 0</td>
<td>1</td>
<td>- -</td>
</tr>
<tr>
<td>0 0.25 0</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>0.25 0.25 0</td>
<td>311</td>
<td>310 1240</td>
</tr>
<tr>
<td>0.25 0 2.5</td>
<td>786</td>
<td>785 3140</td>
</tr>
</tbody>
</table>

\textsuperscript{a}V, vermiculite; P, HF-treated peat; A, ammonium sulfate. In pretreatment of vermiculite-ammonium sulfate mixture, KCl was added to vermiculite immediately before addition of ammonium sulfate.
The amounts of ammonium fixed by vermiculite during pretreatment of vermiculite-peat and vermiculite-ammonium sulfate mixtures with various reagents are presented in Table 9. These data clearly show that the KOBr pretreatment completely prevented ammonium fixation by vermiculite during pretreatment, and that autoclaving with N KOH or boiling with 0.5 N KOH were almost as effective in preventing fixation of ammonium as the KOBr pretreatment. On the other hand, the H2O2 + KCl pretreatment led to fixation of considerable amounts of ammonium during pretreatment. It should be pointed out that vermiculite has a very high ammonium fixing capacity under moist conditions, so this experiment would have to be considered an extreme test of the effectiveness of the various reagents in preventing ammonium fixation during the pretreatment.

Table 9. Amounts of ammonium-N fixed by vermiculite during pretreatment of vermiculite-peat and vermiculite-ammonium sulfate mixtures

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Me. of K/g. of vermiculite</th>
<th>V + P</th>
<th>V + A</th>
</tr>
</thead>
<tbody>
<tr>
<td>--</td>
<td>0</td>
<td>0</td>
<td>4240</td>
</tr>
<tr>
<td>2 N KCl</td>
<td>200</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>N KOH</td>
<td>50</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>0.5 N KOH</td>
<td>80</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>H2O2 + KCl</td>
<td>1.3</td>
<td>1240</td>
<td>3140</td>
</tr>
<tr>
<td>KOBr</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

aV, vermiculite (0.25 g.); P, HF-treated peat (0.25 g.; 8.4 mg. of N); A, ammonium sulfate (2.5 mg. of N). In pretreatment of vermiculite-ammonium sulfate mixture (V + A), pretreatment reagents were added to vermiculite immediately before addition of ammonium sulfate.
It is shown strikingly in Table 9 that the amount of K⁺ in the pre-treatment solutions used (in me. of K⁺/g. of vermiculite) was inversely related to the amount of ammonium fixed by vermiculite during pretreatment, therefore, it seems that the amount of K⁺ in the pretreatment solution may have some influence in the effectiveness of these solutions to prevent ammonium fixation during pretreatment. In the pretreatment with H₂O₂ + KCl, Schachtschabel had assumed that the amount of KCl used (0.7 me. of K⁺/g. of soil, or 1.3 me. of K⁺/g. of vermiculite) would provide an excess of potassium ions in the pretreatment solution to prevent ammonium fixation by soil. However, in the present study using vermiculite, it is apparent (Table 9) that the quantity of K⁺ added to the H₂O₂ pretreatment solution was not effective in blocking fixation of ammonium completely during this pretreatment. To substantiate these findings, additional tests of the effectiveness of the H₂O₂ pretreatment procedure were made using increasing amounts of K⁺ in the pretreatment solution and various organic and inorganic materials as sources of ammonium-N. The data obtained from these tests are presented in Table 10. It is clear from this table that the amount of ammonium fixed during H₂O₂ pretreatment decreased with increasing amounts of K⁺ added to the pretreatment solution. The treatment using only 1.3 me. of K⁺/g. of vermiculite reduced the amount of ammonium-N fixed by vermiculite during pretreatment to about 50% of that fixed when no KCl was added, and increasing the K⁺ concentration to 3.0 or 4.0 me./g. of vermiculite still did not completely block fixation of ammonium-N during H₂O₂ pretreatment.
Table 10. Effect of KCl on fixation of ammonium-N during H$_2$O$_2$ pretreatment of mixtures of vermiculite and nitrogenous materials

<table>
<thead>
<tr>
<th>Amount of KCl used in pretreatment (mg./g. of vermiculite)</th>
<th>Ammonium-N fixed during pretreatment$^a$ (ug./g. of vermiculite)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V + A</td>
</tr>
<tr>
<td>0</td>
<td>6824</td>
</tr>
<tr>
<td>1.3</td>
<td>3140</td>
</tr>
<tr>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td>4.0</td>
<td>1530</td>
</tr>
</tbody>
</table>

$^a$V, vermiculite (0.25 g.); A, ammonium sulfate (2.5 mg. of N); AL, alanine (0.3 mg. of N); P, HF-treated peat (0.25 g.; 8.4 mg. of N); S, soil 3 (0.6 g.; 0.7 mg. of N). In pretreatment of vermiculite-ammonium sulfate mixture (V + A), pretreatment reagents were added to vermiculite immediately before addition of ammonium sulfate.

To ascertain whether the amount of KCl recommended by Schachtschabel was sufficient to block fixation of ammonium completely during H$_2$O$_2$ pretreatment of soil, samples of soil 3 were treated with H$_2$O$_2$ and varying amounts of KCl, using the H$_2$O$_2$ + KCl pretreatment procedure described in Appendix II. Fixed ammonium-N in the H$_2$O$_2$-pretreated soil was determined as described in the preceding experiment. For comparison of the amount of fixed ammonium in H$_2$O$_2$-pretreated soil with that in a sample of the same soil pretreated with KOBr, which has been shown to be effective in removing organic nitrogen from soils (Tables 3 and 4) and in preventing fixation of ammonium during pretreatment (Table 9), a sample of soil 3 was pretreated with KOBr and shaken with 40 ml. of N$_2$HF:N$_2$HCl.
for 24 hours at room temperature. The ammonium-N released by HF treat-
ment of the KOBr-pretreated soil was determined by the KOH distillation
procedure as described in Appendix III. The ammonium-N fixed by soil 3
during the $H_2O_2$ pretreatment was calculated by subtracting the amount of
fixed ammonium-N in the KOBr-pretreated soil from the amount of fixed
ammonium-N in the $H_2O_2$-pretreated soil, and the results of these calcu-
lations are presented in Table 11. It is apparent that the treatment
using the 0.7 me. of $K^+$/g. of soil recommended by Schachtschabel reduced
the amount of ammonium-N fixed during pretreatment to 25% of that fixed
when no KCl was added and increasing the $K^+$ concentration to 4.3 me./g.
of soil still did not completely block fixation of ammonium during $H_2O_2$
pretreatment.

Table 11. Effect of KCl on fixation of ammonium-N during $H_2O_2$
pretreatment of soil 3

<table>
<thead>
<tr>
<th>Amount of KCl used in pretreatment (me./g. of soil)</th>
<th>Fixed ammonium-N in pretreatment residue ppm. of soil</th>
<th>Ammonium-N fixed during pretreatment$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>329</td>
<td>221</td>
</tr>
<tr>
<td>0.7$^b$</td>
<td>164</td>
<td>56</td>
</tr>
<tr>
<td>4.3</td>
<td>114</td>
<td>6</td>
</tr>
</tbody>
</table>

$^a$Fixed ammonium-N in pretreatment residue minus fixed ammonium-N in untreated soil as determined by KOBr-HF method (108 ppm.).

$^b$Amount used in $H_2O_2$ pretreatment recommended by Schachtschabel (1961b).
The fact that considerable amounts of ammonium-N from organic compounds is fixed by soil minerals during H$_2$O$_2$ pretreatment, especially when potassium is not added (Tables 10 and 11), tends to indicate that Rodrigues' (1954) conclusion that his HF-H$_2$SO$_4$ treatment did not release ammonium-N from organic sources may have been erroneous. It would seem very likely that ammonium-N from organic compounds was fixed during the H$_2$O$_2$ pretreatment performed by Rodrigues, since he did not add any potassium to the pretreatment solution.

Release of Fixed Ammonium during Pretreatments

Another possible error which can occur during the pretreatment of soils for determination of fixed ammonium is that the reagents used for removal of organic nitrogen compounds may also release fixed ammonium. Stevenson (1962) reported that prolonged heating of igneous rocks and primary minerals with N KOH caused release of some fixed ammonium from these minerals. Therefore, in order to investigate this source of error, samples of ammonium-saturated vermiculite were given various pretreatments and the amounts of fixed ammonium remaining in the pretreatment residues were determined.

**Methods**

Samples of ammonium-saturated vermiculite (0.2 g.) were pretreated using the procedures described in Appendix II. Two additional pretreatments were also included in this study. In the pretreatment involving 2 N KCl and KOBr, the procedure used was that employed for pretreatment with 2 N KCl followed by that for pretreatment with KOBr.
In the pretreatment involving H$_2$O$_2$, the procedure used was similar to that employed for pretreatment with H$_2$O$_2$-KCl, except that the addition of KCl was omitted. Each pretreatment residue was shaken with 8 ml. of N$_{\text{HF}}$:N$_{\text{HCl}}$ and 1 drop of octyl alcohol for 24 hours at room temperature. The ammonium-N released by the HF treatment was determined by the KOH distillation procedure described in Appendix III. The fixed ammonium-N content of the ammonium-saturated vermiculite was calculated by subtracting the exchangeable ammonium-N content, as determined by the extraction-distillation method, from the total ammonium-N content of the vermiculite, as determined by the Kjeldahl method for total nitrogen.

Results and discussion

The total ammonium-N content of the ammonium-saturated vermiculite was found to be 120 me. of N/100 g., and the exchangeable ammonium-N content to be 18 me. of N/100 g. Therefore, the fixed ammonium-N content of the ammonium-saturated vermiculite used in this study was 102 me. of N/100 g. The amounts of fixed ammonium-N found in the ammonium-saturated vermiculite after various pretreatments are presented in Table 12, which shows that none of the pretreatments led to lower values for fixed ammonium, thus indicating that none of these released fixed ammonium. The fixed ammonium-N value obtained after the 0.5 N KOH pretreatment of Bremner (1959) was slightly higher than the fixed ammonium content of the vermiculite, but this was within experimental error. However, the increases in fixed ammonium-N found in the H$_2$O$_2$-pretreated samples could not be accounted for by experimental error, indicating that ammonium fixation had taken place during the pretreatments involving H$_2$O$_2$. Addition of KCl, as recommended by Schachtschabel reduced, but did not
completely block fixation of ammonium during the H2O2 pretreatment.

Table 12. Effect of various pretreatments on fixed ammonium in ammonium-saturated vermiculite (ASV)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Fixed ammonium-N in pretreatment residue (me./100 g. of ASV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>--</td>
<td>102&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 N KCl</td>
<td>102</td>
</tr>
<tr>
<td>2 N KCl, KOBr</td>
<td>102</td>
</tr>
<tr>
<td>KOBr</td>
<td>102</td>
</tr>
<tr>
<td>N KOH</td>
<td>102</td>
</tr>
<tr>
<td>0.5 N KOH</td>
<td>103</td>
</tr>
<tr>
<td>H2O2-KCl</td>
<td>105</td>
</tr>
<tr>
<td>H2O2</td>
<td>107</td>
</tr>
</tbody>
</table>

<sup>a</sup>Total ammonium-N content of ASV (120 me./100 g.) minus exchangeable ammonium-N content as determined by extraction-distillation method (18 me./100 g.).

Effect of Pretreatments on Release of Ammonium from Soils by 5 N HF:N HCl

The effect of various pretreatments on the ammonium-N released from soils by one HF treatment was studied to obtain evidence of the error in fixed ammonium-N values which occurred in the pretreatment rather than in the washing, HF treatment, or distillation steps involved in the procedures for determination of fixed ammonium. Soils which varied widely in organic matter content were selected for this study and the 5 N HF:N
HCl treatment was used for release of ammonium from soils (work reported in Section VI indicated that this treatment effected practically quantitative release of fixed ammonium from soils).

**Methods**

Samples of soil (1.0 g.) were pretreated by the various procedures described in Appendix II. Each pretreatment residue was shaken with 15 ml. of 5 N HF:N HCl for 24 hours at room temperature and the ammonium-N in the HF-soil mixture was determined by the KOH distillation procedure described in Appendix III. A separate sample of each of the pretreated-soils was treated with a mixture of 12 ml. of 10 N KOH, 15 ml. of 5 N HF:N HCl and 50 ml. of water and the ammonium-N was released by steam distillation of the soil-ca. 0.3 N KOH mixture as described in Appendix III.

**Results and discussion**

The amounts of ammonium-N released by treatment of soils with 5 N HF:N HCl after various pretreatments are shown in Table 13. It is seen that the amount of ammonium in a soil was different after different pretreatments, and the amounts of ammonium released from soils after pretreatments with 0.5 N KOH and H₂O₂-KCl were appreciably higher than those released after pretreatments with KOBr and N KOH, whereas extremely high amounts of ammonium were released from soils after 2 N KCl pretreatment or from unpretreated soils. These large discrepancies in the amount of ammonium in a soil after various pretreatments may be partially accounted for by the amount of ammonium-N released from the pretreatment residue by distillation with ca. 0.3 N KOH (Table 14). It is apparent from this table that extensive release of ammonium from organic nitrogen
Table 13. Amounts of ammonium-N released by treatment of soils with 5 N HF:N HCl (15 ml./g. of soil) after various pretreatments

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>9</th>
<th>10</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>KOBr</td>
<td>155</td>
<td>124</td>
<td>265</td>
<td>179</td>
<td>217</td>
<td>271</td>
<td>202</td>
</tr>
<tr>
<td>N KOH</td>
<td>154</td>
<td>126</td>
<td>271</td>
<td>178</td>
<td>219</td>
<td>270</td>
<td>203</td>
</tr>
<tr>
<td>0.5 N KOH</td>
<td>186</td>
<td>157</td>
<td>279</td>
<td>189</td>
<td>230</td>
<td>276</td>
<td>219</td>
</tr>
<tr>
<td>H2O2-KCl</td>
<td>233</td>
<td>161</td>
<td>282</td>
<td>190</td>
<td>240</td>
<td>279</td>
<td>231</td>
</tr>
<tr>
<td>2 N KCl</td>
<td>683</td>
<td>438</td>
<td>430</td>
<td>286</td>
<td>280</td>
<td>300</td>
<td>403</td>
</tr>
<tr>
<td>--</td>
<td>687</td>
<td>443</td>
<td>405</td>
<td>285</td>
<td>287</td>
<td>314</td>
<td>404</td>
</tr>
</tbody>
</table>

Table 14. Amounts of ammonium-N released by distillation of soils with ca. 0.3 N KOH after various pretreatments

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>9</th>
<th>10</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>KOBr</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N KOH</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0.5 N KOH</td>
<td>23</td>
<td>15</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>H2O2-KCl</td>
<td>64</td>
<td>24</td>
<td>13</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>2 N KCl</td>
<td>506</td>
<td>298</td>
<td>137</td>
<td>87</td>
<td>47</td>
<td>21</td>
<td>183</td>
</tr>
<tr>
<td>--</td>
<td>508</td>
<td>301</td>
<td>120</td>
<td>90</td>
<td>52</td>
<td>31</td>
<td>184</td>
</tr>
</tbody>
</table>

Compounds occurred during the KOH distillation of soils which were unpretreated or pretreated with 2 N KCl, and some release of ammonium from alkali-labile organic nitrogen compounds also occurred during KOH distillation of soils pretreated with 0.5 N KOH and H2O2-KCl. The
N KOH pretreated soils were found to release very small amounts of ammonium from alkali-labile organic nitrogen compounds during KOH distillation, whereas no ammonium was released from alkali-labile organic compounds during KOH distillation of the KOBr-pretreated soils. Realizing that significant breakdown of organic nitrogen compounds could occur when soils pretreated with 0.5 N KOH were distilled with strong alkali, Bremner (1959) had recommended the use of sodium borate buffer (pH 8.8) for distillation to eliminate this source of error.

Attention may be drawn to the fact that if the amounts of ammonium-N released by treatment of soils with 5 N Hf:N HCl after various pretreatments are corrected (subtracted) for the amounts of ammonium-N released by distillation of pretreated soils with ca. 0.3 N KOH (as shown in Table 15), the results obtained are not greatly different. This indicates that the major sources of error in the methods involving no pretreatment or pretreatments with KOH, KCl, and H₂O₂-KCl is their failure to effect complete removal of alkali-labile organic nitrogen compounds.

Table 15. Ammonium-N released by treatment of soils with 5 N Hf:N HCl after various pretreatments corrected for ammonium-N released by distillation of pretreated soils with ca. 0.3 N KOH

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Soil No. 1</th>
<th>Soil No. 2</th>
<th>Soil No. 4</th>
<th>Soil No. 7</th>
<th>Soil No. 9</th>
<th>Soil No. 10</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>KOBr</td>
<td>155</td>
<td>124</td>
<td>265</td>
<td>179</td>
<td>217</td>
<td>271</td>
<td>202</td>
</tr>
<tr>
<td>N KOH</td>
<td>150</td>
<td>124</td>
<td>271</td>
<td>178</td>
<td>219</td>
<td>270</td>
<td>202</td>
</tr>
<tr>
<td>0.5 N KOH</td>
<td>163</td>
<td>142</td>
<td>274</td>
<td>186</td>
<td>229</td>
<td>275</td>
<td>211</td>
</tr>
<tr>
<td>H₂O₂-KCl</td>
<td>169</td>
<td>137</td>
<td>269</td>
<td>178</td>
<td>232</td>
<td>277</td>
<td>210</td>
</tr>
<tr>
<td>2 N KCl</td>
<td>177</td>
<td>140</td>
<td>293</td>
<td>199</td>
<td>233</td>
<td>279</td>
<td>220</td>
</tr>
<tr>
<td>--</td>
<td>179</td>
<td>142</td>
<td>285</td>
<td>195</td>
<td>235</td>
<td>283</td>
<td>220</td>
</tr>
</tbody>
</table>
Summary and Conclusions

It has been the objective of the studies reported in this section to develop an efficient pretreatment procedure for the determination of fixed ammonium in soils and to evaluate this procedure in comparison with other pretreatments used in current HF methods for fixed ammonium. The efficiencies of the pretreatment solutions in removing organic nitrogen were studied, and various sources of error in the pretreatment procedures were investigated.

It has been found that the H$_2$O$_2$-KCl pretreatment procedure of the Schachtschabel method removed about 87% of the organic nitrogen from soils, but that extensive fixation by soil minerals of ammonium released by oxidation of organic nitrogen compounds during the pretreatment occurred. The determination of fixed ammonium following this pretreatment was found to be subject to considerable interference by organic nitrogen compounds as a result of the HF release of ammonium from organic nitrogen compounds during alkaline distillation.

The 0.5 N KOH pretreatment procedure recommended by Bremner did not lead to noticeable fixation of ammonium during the pretreatment or to release of fixed ammonium from vermiculite. Some interference due to release of ammonium from organic nitrogen compounds during KOH distillation was observed in the determination of fixed ammonium following this pretreatment.

The N KOH pretreatment procedure developed by Dhariwal and Stevenson removed about 86% of the organic nitrogen from soils and the determination of fixed ammonium following this pretreatment was not subject to interference by organic nitrogen compounds in soils with less
than 5% organic carbon, but slight interference occurred in soils with 5% or more organic carbon. Furthermore, practically no fixation of ammonium by vermiculite was observed and no release of fixed ammonium from vermiculite occurred during this pretreatment.

The KOBr pretreatment developed in the present study was found to effect almost quantitative (96 to 100%) removal of organic nitrogen under conditions which cause no release of fixed ammonium from vermiculite and involve no risk of fixation during this pretreatment. Determination of fixed ammonium following this pretreatment was not subject to interference by organic nitrogen compounds.
SECTION VI. EVALUATION OF HF TREATMENTS FOR RELEASE OF FIXED AMMONIUM

Introduction

The objectives of evaluating HF treatments are to study the effectiveness of these treatments for release of fixed ammonium and to investigate the possibility of interference from decomposition of organic nitrogen compounds during these treatments. Several recovery studies have been reported on some of the current HF methods. Both Dhariwal and Stevenson (1958) and Bremner and Harada (1959) tested their HF treatments and found that they effected complete recovery of fixed ammonium from vermiculite and from other clay minerals. Bremner (1959) further tested his HF solution on several soils and found that for most surface soils, 20 ml. of _HF: N HCl per g. of soil effected complete release of fixed ammonium. However, the amount of fixed ammonium released from three subsoils continued to increase with increasing amounts of HF solution used above 20 ml. per g. and he therefore, recommended using 40 ml. of the HF solution per g. of soil. Rodrigues (1954) and Schachtschabel (1961b) did not report studies of the efficiencies of their HF treatments for release of fixed ammonium. In this study, tests were performed on the recoveries of fixed ammonium in vermiculite and in soils using varied volume and strength of HF solutions. Also the effect of heating the HF extract, as recommended in some procedures was investigated.

Effect of Heating HF Extract

Two of the current HF methods for determination of fixed ammonium in soils employ heating of the HF extract of soils in the presence of
H₂SO₄ either to drive off the residual HF in the extract (the Rodrigues method) or to effect complete release of fixed ammonium from soil (the Schachtschabel method). The Schachtschabel method includes pretreatment of soils to remove most of the organic nitrogen and has provision to correct for interference from the organic nitrogen not removed by pretreatment; therefore, the error involved in heating the HF extract by this method should be greatly minimized.

The Rodrigues procedure of HF treatment was selected for study to determine the magnitude of error involved in heating the HF extract. A detailed description of the Rodrigues' procedure for determining fixed ammonium is found in Appendix I. For comparison, aliquots of HF extracts obtained by this procedure were also distilled without preheating to drive off the residual HF. This distillation of the HF extract without preheating was performed by transferring an aliquot of the extract to a distillation flask containing 4 ml. of 10 N NaOH through a long-stemmed polyethylene funnel which had its tip under the surface of the NaOH solution. After swirling the flask to mix the contents and allowing it to stand for a few minutes, it was attached to the steam distillation apparatus described in Section VII (Figure 2) and the ammonium was determined by distillation as described in Appendix III. Preliminary studies had shown that complete recovery of ammonium was obtained when a known amount of ammonium was distilled from an HF solution under alkaline conditions by this technique.

The data presented in Table 16 showed that a marked difference in the values for fixed ammonium was obtained between samples preheated to drive off HF and those not preheated. It was observed that Rodrigues'

procedure involving heating of the HF extract with $H_2SO_4$ to drive off the residual HF was very similar to the acid digestion for total nitrogen in the Kjeldahl method. Digestion of organic nitrogen compounds would be expected to take place in this procedure because it was necessary to drive off the water and to boil the $H_2SO_4$ before the residual HF was driven from the extract. During the heating, the sample actually showed a clearing, changing from black or dark brown to light brown or white. The heating obviously had caused extensive release of ammonium from organic compounds in the extract to result in such marked difference between heating and not heating the extract, because it was shown that distillation would completely release fixed ammonium from HF extracts without preheating the extract to drive off HF (Bremner, 1959). Hanway (1954), Hanway and Scott, (1956) and Leggett (1958) modified the Rodrigues method for determination of fixed ammonium by distilling the HF extract without prior heating to drive off HF.

Table 16. Effect of heating HF extract in Rodrigues method of determining fixed ammonium

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Fixed ammonium-N (ppm. of soil)$^a$</th>
<th>H</th>
<th>NH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1460</td>
<td>346</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1260</td>
<td>319</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1150</td>
<td>385</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>735</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>554</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>341</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>917</td>
<td>282</td>
<td></td>
</tr>
</tbody>
</table>

$^a$H, extract was heated before distillation; NH, extract was distilled without prior heating.
Release of Fixed Ammonium from Vermiculite

Several studies have been reported on the release of fixed ammonium by treatment of ammonium-saturated vermiculite with various HF solutions. Dhariwal and Stevenson (1958) compared recoveries of ammonium using five HF solutions ranging in strength from $N\text{ HF}:N\text{ HCl}$ to $20\text{ N HF}:3\text{ N HCl}:2.4\text{ N }\text{H}_2\text{SO}_4$. Bremner and Harada (1959) studied the ammonium recovery from vermiculite using increasing quantities of $N\text{ HF}:N\text{ HCl}$. Walsh and Murdock (1960) also evaluated recovery of ammonium from vermiculite by several of the solutions used by Dhariwal and Stevenson, but used much larger quantities of solution. In the present work, the ammonium recovery from vermiculite was studied using increasing quantities of $N\text{ HF}:N\text{ HCl}$. Data from these studies are summarized in Table 17, and it can be seen that in each study complete recovery of fixed ammonium from ammonium-saturated vermiculite was achieved when the amount of HF used exceeded 40 Me./g. of vermiculite.

Release of Fixed Ammonium from Soils

It has been assumed by most researchers studying fixed ammonium that the amount of HF which gives complete recovery of fixed ammonium in vermiculite will also be sufficient to release all of the fixed ammonium from soils. This assumption was tested on the basis that if all the fixed ammonium in a soil is released by a given treatment with HF solution (i.e., a maximum value for fixed ammonium in this soil is obtained), then an increase in the volume or strength of this solution or in the period of treatment will not increase the fixed ammonium value obtained. An experiment was designed to study the effect of the volume...
Table 17. Comparison of recoveries of fixed ammonium obtained by treatment of ammonium-saturated vermiculite (ASV) with various HF solutions

<table>
<thead>
<tr>
<th>Reference</th>
<th>Normality</th>
<th>Period of shaking (hr.)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Me. of HF/g. of ASV</th>
<th>Recovery of fixed ammonium (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dhariwal and Stevenson</td>
<td>1.0</td>
<td>15</td>
<td>16A</td>
<td>15</td>
</tr>
<tr>
<td>(1958)</td>
<td>2.0</td>
<td>15</td>
<td>16A</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>15</td>
<td>16A</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>15</td>
<td>16A</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>15</td>
<td>16A</td>
<td>300</td>
</tr>
<tr>
<td>Bremner and Harada</td>
<td>1.0</td>
<td>20</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>(1959)</td>
<td>1.0</td>
<td>40</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>Present work</td>
<td>1.0</td>
<td>40</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>60</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>80</td>
<td>24</td>
<td>80</td>
</tr>
<tr>
<td>Walsh and Murdock</td>
<td>1.0</td>
<td>100</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>(1960)</td>
<td>2.0</td>
<td>100</td>
<td>24</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>100</td>
<td>24</td>
<td>500</td>
</tr>
</tbody>
</table>

<sup>a</sup>A, treatment was performed with occasional shaking; all other treatments were performed with continuous shaking.
and strength of HF solutions used, the period of treatment, and the method of shaking the soil-HF mixture on the release of fixed ammonium from soils. The soils used had been pretreated with N KOH in an autoclave and found to be relatively free from interference by organic nitrogen in the determination of fixed ammonium.

Methods

A sample of N KOH-pretreated soil equivalent to 1 g. of untreated soil was used in each HF treatment indicated in Table 18. The ammonium released by HF treatment was determined by the KOH distillation procedure described in Appendix III. A modification of this distillation procedure was used when the volume of the HF-soil mixture was small in that a 100-ml. distillation flask was used and 60 ml. of distillate were collected; when the volume of the HF-soil mixture was large, a 250-ml. distillation flask was used (as described in Appendix III) and 120 ml. of distillate were collected.

Results and discussion

Results of the various treatments are summarized in Table 18. The data obtained by treatments 3 to 7, which are also plotted in Figure 1, clearly show that a minimum of 15 ml. of 5 N HF:0.75 N HCl:0.6 N H₂SO₄ (75 me. of HF/g. of soil) was needed to obtain maximum release of ammonium from all three soils and that an increased quantity of HF or period of treatment did not increase the amount of ammonium released. The data for treatments 1 to 4 show that the treatment recommended by Dhariwal and Stevenson (No. 1) does not effect quantitative release of fixed ammonium, and that this is due to inadequacies in the method of shaking (cf. treatments 1 and 2), the period of treatment (cf. treatments
Table 18. Ammonium-N released from KOH-pretreated soils by various HF treatments

<table>
<thead>
<tr>
<th>No.</th>
<th>HF solution</th>
<th>Ml. of solution/g. of soil</th>
<th>Period of shaking (hr.)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Me. of HF/g. of soil</th>
<th>Soil No.</th>
<th>Ammonium-N released (ppm. of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0 N HF:0.75 N HCl:0.6 N H₂SO₄</td>
<td>7.5</td>
<td>16A</td>
<td>37.5</td>
<td>2</td>
<td>112</td>
</tr>
<tr>
<td>2</td>
<td>5.0 N HF:0.75 N HCl:0.6 N H₂SO₄</td>
<td>7.5</td>
<td>16</td>
<td>37.5</td>
<td>7</td>
<td>121</td>
</tr>
<tr>
<td>3</td>
<td>5.0 N HF:0.75 N HCl:0.6 N H₂SO₄</td>
<td>7.5</td>
<td>24</td>
<td>37.5</td>
<td>9</td>
<td>126</td>
</tr>
<tr>
<td>4</td>
<td>5.0 N HF:0.75 N HCl:0.6 N H₂SO₄</td>
<td>15.0</td>
<td>24</td>
<td>75.0</td>
<td>2</td>
<td>129</td>
</tr>
<tr>
<td>5</td>
<td>5.0 N HF:0.75 N HCl:0.6 N H₂SO₄</td>
<td>30.0</td>
<td>24</td>
<td>150.0</td>
<td>4</td>
<td>130</td>
</tr>
<tr>
<td>6</td>
<td>5.0 N HF:0.75 N HCl:0.6 N H₂SO₄</td>
<td>40.0</td>
<td>24</td>
<td>200.0</td>
<td>6</td>
<td>130</td>
</tr>
<tr>
<td>7</td>
<td>5.0 N HF:0.75 N HCl:0.6 N H₂SO₄</td>
<td>40.0</td>
<td>48</td>
<td>200.0</td>
<td>8</td>
<td>130</td>
</tr>
<tr>
<td>8</td>
<td>1.0 N HF:1.0 N HCl</td>
<td>--</td>
<td>24</td>
<td>40.0</td>
<td>2</td>
<td>113</td>
</tr>
<tr>
<td>9</td>
<td>1.0 N HF:1.0 N HCl</td>
<td>--</td>
<td>24</td>
<td>80.0</td>
<td>7</td>
<td>123</td>
</tr>
<tr>
<td>10</td>
<td>5.0 N HF</td>
<td>--</td>
<td>24</td>
<td>75.0</td>
<td>8</td>
<td>125</td>
</tr>
<tr>
<td>11</td>
<td>10.0 N HF</td>
<td>--</td>
<td>24</td>
<td>150.0</td>
<td>9</td>
<td>129</td>
</tr>
<tr>
<td>12</td>
<td>5.0 N HF:1.0 N HCl</td>
<td>--</td>
<td>24</td>
<td>75.0</td>
<td>10</td>
<td>128</td>
</tr>
<tr>
<td>13</td>
<td>5.0 N HF:1.0 N HCl</td>
<td>--</td>
<td>24</td>
<td>100.0</td>
<td>11</td>
<td>130</td>
</tr>
<tr>
<td>14</td>
<td>5.0 N HF:1.0 N HCl</td>
<td>--</td>
<td>24</td>
<td>150.0</td>
<td>12</td>
<td>130</td>
</tr>
<tr>
<td>15</td>
<td>5.0 N HF:1.0 N HCl</td>
<td>--</td>
<td>48</td>
<td>150.0</td>
<td>13</td>
<td>130</td>
</tr>
</tbody>
</table>

<sup>a</sup>A treatment was performed with occasional shaking; all other treatments were performed with continuous shaking.
Figure 1. Ammonium-N released by shaking KOH-pretreated soils with 5 N HF:0.75 N HCl:0.6 N H2SO4 for 24 hours
2 and 3), and the amount of HF solution (cf. treatments 3 and 4). It is pertinent in this connection to point out that Young and Cattani (1962) found that with the HF solution recommended by Dhariwal and Stevenson, the use of continuous shaking for 14 hours gave more reproducible results than occasional shaking for 16 hours. It should also be noted that the fixed ammonium value obtained from soil 9 (Table 16) using the Rodrigues HF treatment (involving treating soil with 40% HF, or 96 me. of HF/g. of soil, for 1 hour), but without heating of the extract to drive off HF prior to distillation (as described for the results reported in Table 16) was lower than the fixed ammonium content of this soil determined by the HF treatments shown in Table 18. This incomplete release of ammonium by the 96 me. of HF/g. of soil in the Rodrigues method may be due to the relatively short period of treatment with HF (1 hour) and the lack of agitation during HF treatment.

The data for treatments 8 and 9 show that \( \text{N}_4 \text{HF:N}_2 \text{HCl} \) (the reagent used by Bremner) failed to effect complete release of fixed ammonium even when the amount of HF used was 80 me/g. of soil, which exceeds the minimum amount required for complete release of fixed ammonium by more concentrated HF solutions (75 me/g. of soil). The data for treatments 10 to 15 show that HF alone was less effective than the same amount of HF in combination with another acid in releasing fixed ammonium. Although treatment 12 effected practically quantitative release of fixed ammonium, treatment 13, involving the use of 20 ml. of \( 5 \text{N}_4 \text{HF:N}_2 \text{HCl} \) and continuous shaking for 24 hours, would be a more desirable treatment to use.

Apparently complete consumption of HF is not the cause of incomplete release of fixed ammonium. Evidence which verified this was obtained in
separate studies which were conducted to determine the quantity of H+ in HF:HC1 solutions consumed in the HF treatment of soil for 24 hours. It was found that when 40 ml. of N HF:N HCl solution were added per g. of soil (80 me. of H+ per g.), 12 to 18% of the added H+ was consumed, and when 15 ml. of 5 N HF:N HCl were added per g. of soil (90 me. of H+ per g.), 3 to 10% of the added H+ was consumed. In the N HF:N HCl solution, 40 me. of HF were added while in the 5 N HF:N HCl solution, 75 me. of HF were added, thus if it is assumed that only H+ from HF was consumed, then there would be a minimum of 25 and 66 me. of HF per g., respectively, left in the two HF solutions. Thus the HF was not completely exhausted.

Summary and Conclusions

It has been shown that heating the HF extract of soil without prior removal of soil organic nitrogen (as recommended by Rodrigues) led to extensive interference in the determination of fixed ammonium by ammonium released from organic sources during the heating.

The assumption that the amount of HF which gives complete recovery of fixed ammonium from vermiculite will also be sufficient to release all of the fixed ammonium from soils was shown to be erroneous by comparison of the recoveries of fixed ammonium from vermiculite and from soils. It has been found that about 40 me. of HF was sufficient to recover the fixed ammonium completely in 1 g. of vermiculite, but a minimum of 75 me. of HF was needed to release all of the fixed ammonium in 1 g. of soil.

The main defects of most of the currently-used HF treatments are due to inadequacies in the method of shaking, the period of treatment, and the amount of HF solution used in the treatment.
SECTION VII. KOBr-HF METHOD OF DETERMINING FIXED AMMONIUM

Introduction

The KOBr pretreatment has been shown to be the most efficient in removing organic nitrogen from soils and in preventing interference by ammonium from organic matter in the determination of fixed ammonium (See Section V). The \( \text{KOH} \) pretreatment of Dhariwal and Stevenson (1958) was less efficient in removing organic nitrogen from soils than the KOBr pretreatment and it did not prevent interference by ammonium from organic nitrogen compounds in soils containing 5 to 10% organic carbon. Therefore, this pretreatment procedure was not selected for the determination of fixed ammonium. The results in Section VI showed that the HF solution consisting of \( 5 \text{ N HF}: \text{N HCl} \) effected complete release of fixed ammonium from \( \text{KOH} \)-pretreated soils when used at the rate of 100 me. of HF/g. of soil. In view of these findings, the combination of the KOBr pretreatment and the \( 5 \text{ N HF}: \text{N HCl} \) solution should provide the best method for determination of fixed ammonium in soils.

The effectiveness of this proposed method in assessing the fixed ammonium content of the inorganic soil constituents was investigated, and the most suitable procedure for the determination of fixed ammonium was developed.

Evaluation of HF Treatments for Release of Fixed Ammonium from KOBr-Pretreated Soils

The HF treatments for release of fixed ammonium from KOBr-pretreated soils were evaluated at room temperature and at 100°C.
Treatments at room temperature

The data presented in Table 18 for the release of ammonium by HF solutions were obtained from N-KOH-pretreated soils; therefore, a similar study was conducted on soils to determine the HF treatment required for complete release of fixed ammonium following the KOBr pretreatment.

Pretreatment  The KOBr pretreatment was performed as described in Appendix II.

HF treatment  Ammonium-N was released from KOBr-pretreatment residues with N HF:N HCl and 5 N HF:N HCl solutions by shaking the HF-soil mixtures for 24 hours and 48 hours at room temperature. The ammonium released by the HF treatments was determined by the KOH distillation procedure as described in Appendix III.

Results and discussion  The data presented in Table 19 confirm the findings in Table 18 that 100 me. of HF/g. of soil, when supplied as 5 N HF:N HCl, effects release of the maximum amount of ammonium from pretreated soils in a 24-hour period of shaking. Increasing the amount of HF added, or the period of shaking did not release any more ammonium from the 6 KOBr-pretreated soils. When only 75 me. of HF/g. of soil was supplied by the 5 N HF:N HCl solution, an average of 99% of the maximum amount of ammonium was released, but increasing the period of treatment to 48 hours, resulted in release of the maximum amount of ammonium. The N HF:N HCl solution recommended by Bremner (1959) supplied 40 me. of HF/g. of soil and released an average of 90% of the maximum amount of ammonium in these KOBr-pretreated soils in a 24-hour period of shaking. Increasing the shaking period to 48 hours resulted in increased release of ammonium, but the average amount released was only 93% of the
Table 19. Ammonium-N released from KOBr-pretreated soils by various HF treatments

<table>
<thead>
<tr>
<th>HF treatment</th>
<th>Ml. of solution/g. of soil</th>
<th>Period of shaking (hr.)</th>
<th>Me. of HF/g. of soil</th>
<th>Soil No.</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Average[^]{^}</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>HF solution</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>N HF:N HCl</td>
<td>40</td>
<td>24</td>
<td>40</td>
<td>144</td>
<td>112</td>
<td>249</td>
<td>166</td>
<td>188</td>
<td>243  (90)</td>
</tr>
<tr>
<td>2</td>
<td>N HF:N HCl</td>
<td>40</td>
<td>48</td>
<td>40</td>
<td>148</td>
<td>118</td>
<td>252</td>
<td>173</td>
<td>202</td>
<td>252  (93)</td>
</tr>
<tr>
<td>3</td>
<td>5 N HF:N HCl</td>
<td>15</td>
<td>24</td>
<td>75</td>
<td>155</td>
<td>124</td>
<td>265</td>
<td>179</td>
<td>217</td>
<td>271  (99)</td>
</tr>
<tr>
<td>4</td>
<td>5 N HF:N HCl</td>
<td>15</td>
<td>48</td>
<td>75</td>
<td>158</td>
<td>124</td>
<td>268</td>
<td>182</td>
<td>217</td>
<td>273  (100)</td>
</tr>
<tr>
<td>5</td>
<td>5 N HF:N HCl</td>
<td>20</td>
<td>24</td>
<td>100</td>
<td>158</td>
<td>124</td>
<td>268</td>
<td>182</td>
<td>217</td>
<td>273  (100)</td>
</tr>
<tr>
<td>6</td>
<td>5 N HF:N HCl</td>
<td>20</td>
<td>48</td>
<td>100</td>
<td>158</td>
<td>124</td>
<td>268</td>
<td>182</td>
<td>217</td>
<td>273  (100)</td>
</tr>
<tr>
<td>7</td>
<td>5 N HF:N HCl</td>
<td>30</td>
<td>24</td>
<td>150</td>
<td>158</td>
<td>124</td>
<td>268</td>
<td>182</td>
<td>217</td>
<td>273  (100)</td>
</tr>
<tr>
<td>8</td>
<td>5 N HF:N HCl</td>
<td>30</td>
<td>48</td>
<td>150</td>
<td>158</td>
<td>124</td>
<td>268</td>
<td>182</td>
<td>217</td>
<td>273  (100)</td>
</tr>
</tbody>
</table>

[^]{^}Figures in parentheses are average values calculated as percentages of the average value for treatment 5.
The maximum amount of ammonium released by 20 ml. of 5 N HF:N HCl solution in a 24-hour period was essentially the same for soils 2, 7 and 9 after KOH and KOBr pretreatments (Tables 18 and 19). The fact that a slightly higher amount of ammonium was released from soil 2 after KOH pretreatment than after KOBr pretreatment may be due to a small amount of ammonium-N released from organic nitrogen compounds remaining in the pretreatment residue, which was shown to occur with this soil (Table 14). Thus it appears that the optimum procedure for the HF treatment in releasing fixed ammonium is to shake the KOBr-pretreated soils continuously with 20 ml. of 5 N HF:N HCl (100 me. of HF/g. of soil) for 24 hours.

**Treatments at 100°C**

Under some circumstances, the method for determining fixed ammonium which requires a 24-hour period of HF treatment may be considered too slow; therefore, an attempt was made to develop a more rapid method. The possibility that heating the HF-soil mixture would increase the speed of reaction and allow complete release of fixed ammonium in a shorter period of time was investigated by heating mixtures of 5 N HF:N HCl solution and KOBr-pretreated soil in a water bath (100°C.) for different time periods.

**Pretreatment** The KOBr pretreatment was performed as described in Appendix II.

**HF treatment** The 5 N HF:N HCl solution was added to KOBr-pretreated soils in different amounts to supply 75, 100 and 150 me. of HF/g. of soil and the soil-HF mixtures were heated for periods of 0.5, 1.0, 2.0 and 4.0 hours in a water bath (100°C.). The ammonium-N released
by HF treatment was determined by the KOH distillation procedure described in Appendix III.

**Results and discussion** The data presented in Table 20 indicate that the amount of ammonium-N released from soils 2 and 9, after KOBr-pretreatment, by heating with 20 ml. of HF solution (100 me. of HF/g. of soil) for 0.5 hour was nearly the same as the amount released by shaking these soils at room temperature for 24 hours with the same HF solution. Increasing the period of heating or the amount of HF solution did not result in a large increase in the amount of ammonium-N released from these soils. On the other hand, more ammonium-N was released from soil 7 as the amount of HF solution and the period of heating were increased. On the basis of the results obtained with soils 2 and 9, other KOBr-pretreated soils were tested. The soil was heated with 20 ml. of HF solution (100 me. of HF/g. of soil) for 0.5 hour and the amount of ammonium-N released by the HF treatment was determined and presented in Table 21, together with the amount of ammonium-N released by treatment of these KOBr-pretreated soils with the same HF solution for 24 hours at room temperature. Similar amounts of ammonium-N were obtained from soils 4, 9 and 10 by the two procedures, while somewhat higher amounts of ammonium-N were released from soils 1 and 2 by heating with HF solution. The increased release of ammonium-N from soils 1 and 2 may have been due to release of ammonium from organic nitrogen compounds remaining in the residue by the hot HF solution since these soils had 10 and 5% organic carbon, respectively. Only 97% of the ammonium-N released by the HF solution at room temperature was released by the heated HF solution from soil 7. The reason for this low release of ammonium is not readily
Table 20. Amounts of ammonium-N released by treatment of KOBr-pretreated soils with 5 N HF:N HCl at 100°C.

<table>
<thead>
<tr>
<th>HF treatment</th>
<th>Soil No.</th>
<th>Ml. of HF solution/g. of soil</th>
<th>Me. of HF/g. of soil</th>
<th>Period of treatment (hr.)</th>
<th>Period of treatment (hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium-N released (ppm. of soil)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>75</td>
<td></td>
<td>125 (124)</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>100</td>
<td></td>
<td>128 (124)</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>150</td>
<td></td>
<td>129 (124)</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>75</td>
<td></td>
<td>167 (179)</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>100</td>
<td></td>
<td>176 (182)</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>150</td>
<td></td>
<td>177 (182)</td>
<td>-</td>
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<tr>
<td>9</td>
<td>15</td>
<td>75</td>
<td></td>
<td>208 (217)</td>
<td>213</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>100</td>
<td></td>
<td>217 (217)</td>
<td>218</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>150</td>
<td></td>
<td>217 (217)</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Figures in parentheses are amounts of ammonium-N released by shaking KOBr-pretreated soils with 5 N HF:N HCl at room temperature for 24 hours.

Table 21. Amounts of ammonium-N released by treatment of KOBr-pretreated soils with 5 N HF:N HCl at 24°C. and 100°C.

<table>
<thead>
<tr>
<th>Treatment with 5 N HF:N HCl&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Soil No.</th>
<th>24°C., 24 hr.</th>
<th>100°C., 30 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium-N released (ppm. of soil)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>158</td>
<td>161</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>124</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>268</td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>182</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>217</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>273</td>
<td>273</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>204</td>
<td>204</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Used 20 ml. of 5 N HF:N HCl (100 me. of HF)/g. of soil.
apparent. These data indicate that an acceptable estimate of fixed ammonium can be obtained quite rapidly for most soils by heating the KOBr-pretreated soils with 20 ml. of 5 N HF:N HCl solution in a water bath (100°C.) for 0.5 hour. If accurate values for fixed ammonium are required for soils containing 5% or more organic carbon, the HF treatment for 24 hours at room temperature should be used.

Comparison of Distillation Methods for Determination of Ammonium Released by HF Treatments of KOBr-Pretreated Soils

Distillation of HF-KOBr residue mixtures with NaOH and KOH

In the HF methods currently used for determining fixed ammonium, NaOH or sodium borate buffer (pH 8.8) has been used for distillation of the ammonium-N released from soil by HF treatment. Distillation with NaOH was not used in the KOBr-HF method developed in the present study, because a measure of the ammonium-N released from the pretreatment residue by alkaline distillation was desired, and NaOH would cause release of fixed ammonium and give erroneous results. Therefore, KOH distillation was used for liberation of ammonium in the KOBr-HF method. However, since it has been established that ammonium-N is not released from KOBr residues by distillation with KOH (Table 14), an experiment was conducted to determine whether the release of ammonium-N from HF-KOBr residue mixtures was the same by distillation with NaOH and KOH.

Pretreatment

The KOBr pretreatment was performed as described in Appendix II.

HF treatment

Ammonium-N was released from the KOBr-pretreatment residues by shaking with 20 ml. of 5 N HF:N HCl for 24 hours at room temperature.
Distillation Determination of the ammonium-N released by HF treatment was performed as described in Appendix III using NaOH for distillation of one set of samples and using KOH for distillation of the other set of samples. Ninety ml. of distillate were collected in 10 ml. of boric acid-indicator solution in a 250-ml. Erlenmeyer flask and two additional 30-ml. quantities of distillate were collected consecutively in 5 ml. of boric acid-indicator solution in 50-ml. Erlenmeyer flasks to provide a total of 150 ml. of distillate collected from each sample.

Results and discussion The amounts of ammonium-N released from the HF-KOBr residue mixtures by distillation with NaOH and KOH are presented in Table 22 and it is apparent that distillation with either NaOH or KOH released the same total amount of ammonium, but the rate of release of ammonium was much slower by NaOH distillation than by KOH distillation. Complete release of ammonium-N was obtained during the first 12 minutes of distillation (90 ml. of distillate) with KOH, but release of only 98% of the ammonium-N was obtained during the first 12 minutes of distillation (90 ml. of distillate) with NaOH. Extending the period of NaOH distillation to 16 and 20 minutes (120 and 150 ml. of distillate) resulted in the release of additional quantities of ammonium-N and complete release of ammonium-N was obtained after 20 minutes of distillation. The reason for the differential rate of release of ammonium by these two bases is not readily apparent. Considerable research would have to be done to determine the mechanism of release of ammonium-ions from solutions during steam distillation of HF-KOBr residue mixtures with various bases to resolve this question. On the basis of
these results, it was decided to adopt distillation with KOH for the release of ammonium-N from HF-KOBr residue mixtures in the final procedure.

Table 22. Amounts of ammonium-N liberated by alkali distillation of mixtures obtained by treatment of KOBr-pretreated soils with 5 N HF:N HCl (20 ml./g. of soil)

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Alkali&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Volume of distillate collected (ml.)</th>
<th>Ammonium-N in distillate (ppm. of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td>1</td>
<td>NaOH</td>
<td>153</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td>158</td>
<td>158</td>
</tr>
<tr>
<td>2</td>
<td>NaOH</td>
<td>123</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td>124</td>
<td>124</td>
</tr>
<tr>
<td>4</td>
<td>NaOH</td>
<td>264</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td>268</td>
<td>268</td>
</tr>
<tr>
<td>7</td>
<td>NaOH</td>
<td>180</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td>182</td>
<td>182</td>
</tr>
<tr>
<td>9</td>
<td>NaOH</td>
<td>212</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td>217</td>
<td>217</td>
</tr>
<tr>
<td>10</td>
<td>NaOH</td>
<td>267</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td>273</td>
<td>273</td>
</tr>
<tr>
<td>Average</td>
<td>NaOH</td>
<td>200</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td>204</td>
<td>204</td>
</tr>
</tbody>
</table>

<sup>a</sup>Concentration of alkali ca. 0.5N.
Distillation of HF-KOBr residue mixtures and HF extracts

Several of the current HF procedures employ direct distillation of the HF-soil mixture with base (Dhariwal and Stevenson, 1958; Schachtschabel, 1961b) while others use distillation of aliquots (filtered or unfiltered) of HF extracts of soil (Rodrigues, 1954; Bremner, 1959) for the determination of ammonium-N released by HF treatment. Although the direct distillation of the HF-soil mixture is faster and requires less handling of HF, there is more danger of decomposition of organic nitrogen compounds during distillation of the HF-soil mixture than during distillation of the filtered HF extract. Therefore, an experiment was conducted to determine whether the same quantity of ammonium was liberated by distillation of a filtered HF extract from a KOBr-residue as by direct distillation of the HF-KOBr residue mixture. The \(\text{N}_\text{HF:}\text{N}_\text{HCl}\) solution of Bremner (1959) was employed in this study to avoid possible loss of ammonium-N in the filtering process, which was reported by Walsh and Murdock (1960). Although this HF solution does not effect complete release of fixed ammonium-N from soils, it will nonetheless allow investigation of the distillation procedure. The three distillation procedures investigated in this study were direct distillation of the HF-KOBr residue with KOH, and distillation of samples of filtered HF extract of the KOBr-residue with KOH and with sodium borate buffer (pH 8.8). The KOBr-residues which were extracted with HF solution were dried prior to HF treatment to facilitate the subsequent volumetric analysis.

Pretreatment The KOBr pretreatment was performed as described in Appendix II. One set of samples was dried over KOH, under vacuum following the 0.5 \(\text{N}_\text{KCl}\) wash.
**HF treatment**  Ammonium-N was released from the KOBr residue by shaking with 40 ml. of $\text{N H}_2\text{HCl}$ (added with a polyethylene graduated cylinder after the 0.5 N KCl wash or added with a pipette after drying over KOH) for 24 hours at room temperature.

**Distillation**  Ammonium-N released by HF treatment of the KOBr residues, which had not been dried before HF treatment, was determined by direct distillation with KOH (ca. 0.5 N) as described in Appendix III with the modification that 120 ml. of distillate were collected.

Ammonium-N released by HF treatment of dried KOBr-residues was determined by distillation of an aliquot of the filtered HF extract. One 15-ml. aliquot of the HF extract was transferred through a long-stemmed polyethylene funnel into a 100-ml. distillation flask containing 4 ml. of 10 N KOH and marked to indicate a volume of 35 ml. The funnel was rinsed with water until the level of liquid in the distillation flask reached the 35-ml. mark, and after having allowed the flask to stand for a few minutes, it was attached to the steam-distillation apparatus described in this section (Figure 2). The ammonium-N released by steam distillation with KOH (ca. 0.5 N) was collected in 5 ml. of boric acid-indicator solution in a 50-ml. Erlenmeyer flask marked to indicate a volume of 35 ml. and 6 consecutive 30-ml. lots of distillate were collected. A second 15-ml. aliquot of the HF extract was transferred to a 100-ml. distillation flask and neutralized by cautious addition of 2 N NaOH, with phenolphthalein as the indicator. At the completion of neutralization, 10 ml. of sodium borate buffer (pH 8.8) were added and after a short period, the ammonium was released by steam distillation. The distillate was also collected in 5 ml. of boric acid-indicator solution and 6 consecutive
30-ml. lots of distillate were obtained.

**Results and discussion** The ammonium-N values determined by the three distillation methods following HF treatment of the KOBr-residues are presented in Table 23 and it is seen that all three distillation procedures gave similar ammonium-N values.

Table 23. Comparison of methods of determining ammonium-N in mixtures obtained by shaking KOBr-pretreated soils with N HF:N HCl (40 ml./g. of soil) for 24 hours

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Methoda</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ammonium-N (ppm. of soil)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>142</td>
<td>143</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>114</td>
<td>113</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>247</td>
<td>249</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>167</td>
<td>167</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>191</td>
<td>190</td>
<td>188</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>240</td>
<td>241</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>184</td>
<td>184</td>
<td>184</td>
<td></td>
</tr>
</tbody>
</table>

aA, mixture was filtered and aliquot of extract was neutralized (NaOH) and distilled with sodium borate buffer (pH 8.8); B, as in A, but aliquot of extract was distilled with KOH (ca. 0.5 N); C, mixture was distilled directly with KOH (ca. 0.5 N).

These results indicate that ammonium-N was not released from organic nitrogen compounds by direct KOH distillation of the HF-KOBr residue mixture or by KOH distillation of the filtered HF extract. Bremner (1959) had shown that the sodium borate buffer distillation procedure released no more ammonium-N from organic nitrogen compounds than the mild
MgO microdiffusion procedure. Therefore, if distillation with KOH had caused significant decomposition of organic nitrogen compounds, considerably higher values for ammonium-N would have been obtained by this distillation procedure than by the sodium borate buffer distillation. These results agreed with the data reported in Table 14.

The rate of release of ammonium-N from the HF extracts which were distilled with sodium borate buffer and with KOH was determined from the amount of ammonium-N in the consecutive 30-ml. lots of distillate collected from each sample. The data presented in Table 24 indicate that the rate of ammonium release by distillation with sodium borate buffer is slower than by distillation with KOH. Complete release of ammonium-N was obtained after distillation with KOH for 16 minutes (120 ml. of distillate) while complete release of ammonium-N was obtained after distillation with sodium borate buffer for 24 minutes (180 ml. of distillate). These results substantiate the data reported in Table 22 which showed that distillation with NaOH released ammonium-N more slowly than distillation with KOH. The pH of the sodium borate buffer mixture was about 8.5 after distillation, while that of the KOH mixture was about 13.0. The data in Tables 22 and 24 suggests that the sodium-ion may be responsible for this differential rate of release of ammonium during distillation and, therefore, care must be taken when sodium compounds are used for distillation of solutions containing HF to verify that complete release of ammonium has been achieved in the selected distillation time.
Table 24. Amounts of ammonium-N released by distillation of \( \text{H}_2\text{O} \cdot \text{HF}:\text{HCl} \)
 extracts of KOBr-pretreated soils with alkaline reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume of distillate collected (ml.)</th>
<th>Ammonium-N (% of maximal amount released by KOH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Na borate (pH 8.8)</td>
<td>86</td>
<td>92</td>
</tr>
<tr>
<td>KOH (ca. 0.5 N)</td>
<td>95</td>
<td>97</td>
</tr>
</tbody>
</table>

\(^a\)Average of results obtained with extracts of six soils.

Preparation of KOBr solution

Since the rate of decomposition of potassium hypobromite solution is reduced if the solution is kept at low temperature, the KOBr solution in the work reported in the previous sections was prepared at low temperature (ca. 4°C.) and used immediately after its preparation. If cooling were not required, preparation of this solution would be simplified; therefore, experiments were conducted to determine if the amount of ammonium-N released by HF treatment after KOBr pretreatment would be affected if the KOBr solution is prepared at room temperature, and used immediately after preparation.

Methods

The KOBr pretreatment was performed as described in Appendix II, with the modifications that one set of samples was treated with KOBr prepared at room temperature and both sets of samples were allowed to stand at room temperature with KOBr for 2, 6, and 10 hours before they
were boiled for 5 minutes. The ammonium-N was released by shaking the KOBr-residues with 20 ml. of 5 N HF:N HCl for 24 hours at room temperature and the ammonium-N released by HF treatment was determined by distillation with KOH as described in Appendix III.

Results and discussion

The amount of ammonium-N released from soils 1 and 5 by HF treatment following pretreatment with KOBr prepared at room temperature and at low temperature are presented in Table 25 and it is readily apparent that the method of KOBr preparation had no effect on the ammonium-N released by HF treatment. Therefore, it was decided to adopt the method of preparation of KOBr which does not require cooling.

Table 25. Effect of method of preparing KOBr solution on amount of ammonium-N released by shaking KOBr-pretreated soil with 5 N HF:N HCl (20 ml./g. of soil) for 24 hours

<table>
<thead>
<tr>
<th>Pretreatment KOBr solutiona</th>
<th>Period (hr.)b</th>
<th>Ammonium-N released (ppm. of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Soil 1</td>
</tr>
<tr>
<td>A</td>
<td>2.0</td>
<td>159</td>
</tr>
<tr>
<td>B</td>
<td>2.0</td>
<td>158</td>
</tr>
<tr>
<td>A</td>
<td>6.0</td>
<td>158</td>
</tr>
<tr>
<td>B</td>
<td>6.0</td>
<td>158</td>
</tr>
<tr>
<td>A</td>
<td>10.0</td>
<td>157</td>
</tr>
<tr>
<td>B</td>
<td>10.0</td>
<td>158</td>
</tr>
</tbody>
</table>

aA, solution was prepared at low temperature (ca. 4° C.); B, solution was prepared at room temperature (ca. 23°C.).

bSoil-KOBr mixture was boiled vigorously for 5 minutes after standing at room temperature for period indicated.
Description of KOBr-HF Method

On the basis of the experiments reported in this dissertation, two procedures were adopted for the determination of fixed ammonium. These procedures involve pretreatment of the sample with KOBr solution and treatment of the residue with HF-HCl solution. In procedure A, the acid treatment is performed by shaking the pretreatment residue with 5 N HF: N HCl for 24 hours at room temperature. In procedure B, this treatment is performed by heating the pretreatment residue with 5 N HF: N HCl at 100°C. for 30 minutes.

Apparatus

Steam-distillation apparatus The apparatus used (Figure 2) is designed so that flasks fitted with standard-taper (19/38) ground-glass joints can be used as distillation chambers. The steam required for distillation is generated by heating distilled water in a 5-liter flask which contains pumice or glass beads (to promote smooth boiling) and about 3 ml. of concentrated sulfuric acid (to trap any ammonium in the distilled water). Before use, the apparatus should be steamed out for about 10 minutes to remove traces of ammonia, and the rate of steam generation adjusted so that 7 to 8 ml. of distillate are collected per minute. The flow of cold water through the condenser of the apparatus should be such that the temperature of the distillate obtained using this rate of distillation does not exceed 22°C. The desired rate of distillation is readily obtained if the steam generator flask is heated by an electric heating mantle and the power supply to the mantle is controlled by a variable transformer. The trap at the base of the water jacket on the condenser is to prevent any water condensing on the
Figure 2. Steam-distillation apparatus
external surface of the condenser from entering the flask used to collect the distillate.

**Distillation flasks** The flasks used are 250-ml. Kjeldahl flasks fitted with standard-taper (19/38) ground-glass joints and glass hooks so that they can be connected to the steam-distillation apparatus by spiral steel springs with loop ends. Their dimensions should be such that when the flasks are connected to the steam distillation apparatus, the distance between the tip of the steam inlet tube and the bottom of the flask is approximately 4 mm.

**Reagents**

**Potassium hypobromite solution** Prepare this reagent immediately before use by addition of 6 ml. of bromine to 200 ml. of approximately 2 N KOH. Add the bromine slowly (ca. 0.5 ml./min.) and with constant stirring.

**Potassium chloride solution (ca. 0.5 N)** Dissolve 186 g. of KCl in 5 liters of water.

**Hydrofluoric acid-hydrochloric acid solution (ca. 5 N HF: N HCl)** Place about 1500 ml. of water in a 2.5-liter polyethylene or polypropylene bottle marked to indicate a volume of 2 liters, and add 167 ml. of concentrated hydrochloric acid (sp. gr. 1.19) and 325 ml. of approximately 52% (w/w) hydrofluoric acid (ca. 31 N). Then dilute the solution to the 2-liter mark, and mix it thoroughly.

**Potassium hydroxide solution (ca. 10 N)** Dissolve 2800 g. of KOH in approximately 4 liters of water, and dilute the cooled solution to a volume of 5 liters. Store the solution in a tightly stoppered bottle.
Boric acid-indicator solution  Dissolve 20 g. of reagent grade boric acid in about 700 ml. of hot water, and transfer the cooled solution to a 1-liter volumetric flask containing 220 ml. of mixed indicator solution prepared by dissolving 0.060 g. of bromocresol green and 0.030 g. of methyl red in 1000 ml. of 95% ethanol. After mixing the contents of the flask, add approximately 0.05 N NaOH cautiously until a color change from pink to pale green is just detectable when 1 ml. of the solution is treated with 1 ml. of water. Then dilute the solution to volume with water, and mix it thoroughly.

Sulfuric acid  0.005 N standard.

Procedures

Procedure A  Place a 1-g. sample of finely ground (<100 mesh) soil in a 200-ml. tall-form beaker, and add 20 ml. of potassium hypobromite solution. Swirl the beaker to mix the soil and hypobromite, and then allow it to stand with its mouth covered by a watch glass. After 2 hours, add 60 ml. of water, and heat the covered beaker on an electric hot plate until the soil-hypobromite mixture has boiled vigorously for 5 minutes. Allow the mixture to cool and settle in the covered beaker (preferably overnight), and decant and discard the clear supernatant liquid. Transfer the residue with 0.5 N KCl to a 100-ml. polyethylene or polypropylene centrifuge tube marked to indicate a volume of 80 ml. Use a wash bottle containing 0.5 N KCl to perform this transfer, and bring the contents of the tube to the 80-ml. mark with the washings obtained by rinsing the beaker several times with 0.5 N KCl. Fit the neck of the tube with a polyethylene cap or rubber stopper, and, after shaking the tube manually for a few seconds, centrifuge it at 2000 rpm.
(ca. 1000 x g.) for 10 minutes. Decant the clear supernatant liquid, add 0.5 N KCl to the 80-ml. mark, and shake and centrifuge the tube as in the previous operation. Decant the clear supernatant liquid, and add 20 ml. of 5 N HF:N HCl solution (from a 25-ml. polyethylene graduated cylinder). Then stopper the tube, and shake it for 24 hours on a mechanical shaker.

After completion of the treatment with HF-HCl solution, add 15 ml. of 10 N KOH to a distillation flask marked to indicate a volume of 60 ml., and place a long-stemmed polyethylene funnel in the neck of the flask so that the end of its stem is below the surface of the KOH solution. Transfer the contents of the centrifuge tube to the distillation flask through this funnel, and complete the transfer by rinsing the centrifuge tube and funnel with water until the level of the liquid in the flask reaches the 60-ml. mark. Then stopper the flask, swirl it to mix the contents, and allow it to stand for a few minutes. During this period of standing, add 10 ml. of boric acid-indicator solution to a 250-ml. Erlenmeyer flask marked to indicate a volume of 100 ml., and place the flask under the condenser of the distillation apparatus so that the end of the condenser is about 4 cm. above the surface of the boric acid. Attach the distillation flask to the distillation apparatus by spiral steel springs as shown in Figure 2, and immediately commence distillation by closing the stopcock on the steam by-pass tube of the distillation apparatus. When the distillate reaches the 100-ml. mark on the receiver flask, stop the distillation by opening the stopcock on the steam by-pass tube (period of distillation, ca. 12 minutes). Then rinse the end of the condenser, and determine ammonium-N in the distillate by titration.
with 0.005 N sulfuric acid from a 5-ml. microburette graduated at 0.01-ml. intervals (1 ml. 0.005 N H₂SO⁴ = 70 μg. NH -N). The color change at the end-point is from green to a faint, permanent pink. To calculate fixed ammonium-N in the sample, determine the amount of ammonium-N liberated by steam distillation of 20 ml. of 5 N HF: N HCl solution with 15 ml. of 10 N KOH solution, and subtract the ammonium-N value obtained in this analysis from that obtained in analysis of the sample. In performing this control analysis, follow the procedure described for determination of the ammonium released by treatment of the soil sample with HF-HCl solution.

**Procedure B**  
Treat the soil sample (1 g.) with potassium hypobromite solution and wash the residue from this treatment with 0.5 N KCl as described in procedure A. Then add 20 ml. of 5 N HF: N HCl to the washed residue in the centrifuge tube, and swirl the tube until the contents are thoroughly mixed. (Mixing can be facilitated by tapping the bottom of the tube on the bench at intervals during swirling). After mixing, cover the mouth of the tube with an inverted 100-ml. polyethylene beaker, and place the tube in a vigorously boiling water bath (situated in a hood) so that the lower half of the tube is completely immersed in boiling water. After about 5 minutes, remove the tube from the bath and swirl it for a few seconds to thoroughly mix the contents. If any large aggregates of soil particles are observed, break them up with a polyethylene stirring rod and rinse the rod with a little water before removing it from the tube. Return the tube to the water bath, and, at approximately 10-minute intervals, remove the tube from the bath, swirl it for a few seconds, and return it to the bath. After
heating the tube for a total of 30 minutes, remove it from the bath, swirl it for a few seconds, and allow it to cool for about 1 hour. Add 15 ml. of 10 N KOH to a distillation flask marked to indicate a volume of 60 ml., and place a long-stemmed polyethylene funnel in the neck of the flask so that the end of its stem is below the surface of the KOH solution. Transfer the contents of the centrifuge tube to the distillation flask through this funnel, and complete the transfer by rinsing the tube and funnel with water until the level of the liquid in the flask reaches the 60-ml. mark. Then stopper the flask, swirl it to mix the contents, and, after allowing it to stand for a few minutes, connect it to the distillation apparatus and determine the amount of ammonium liberated by steam distillation as described in Procedure A. To calculate fixed ammonium in the sample, determine the amount of ammonium liberated by steam distillation of 20 ml. of 5 N HF: N HCl with 15 ml. of 10 N KOH, and subtract the ammonium-N value obtained in this analysis from that obtained in analysis of the sample. In performing this control analysis, follow the procedure described for determination of the ammonium released by treatment of the soil sample with HF-HCl solution.

Precision of KOBr-HF Method

Fixed ammonium-N values were obtained from 7 soils by the KOBr-HF method (Procedure A) and the range, the mean of 8 determinations, the standard deviation and the coefficient of variation for the individual soils are presented in Table 26. The standard deviations of the individual soils range from 0.6 to 1.4 ppm. of fixed ammonium-N which indicates excellent precision for all 7 soils. The overall standard deviation
obtained for the 7 soils was 1.1 ppm. of fixed ammonium-N and shows that this method is considerably more precise than the N KOH-HF method proposed by Dhariwal and Stevenson for which they reported a standard deviation of 0.04 me. of fixed ammonium-N/100 g. of soil or 5.6 ppm. of fixed ammonium-N.

Table 26. Precision of KOBr-HF method (Procedure A)

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Range</th>
<th>Mean</th>
<th>Sb</th>
<th>CVc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>157-159</td>
<td>158</td>
<td>0.71</td>
<td>0.45</td>
</tr>
<tr>
<td>2</td>
<td>123-125</td>
<td>124</td>
<td>0.65</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
<td>107-109</td>
<td>108</td>
<td>0.62</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>266-269</td>
<td>268</td>
<td>1.30</td>
<td>0.48</td>
</tr>
<tr>
<td>8</td>
<td>91-93</td>
<td>92</td>
<td>0.58</td>
<td>0.63</td>
</tr>
<tr>
<td>9</td>
<td>216-219</td>
<td>217</td>
<td>1.24</td>
<td>0.57</td>
</tr>
<tr>
<td>10</td>
<td>270-274</td>
<td>273</td>
<td>1.41</td>
<td>0.52</td>
</tr>
</tbody>
</table>

^aEight determinations.
^bStandard deviation.
^cCoefficient of variation.

The good precision of the KOBr-HF method results from the fact that essentially all interference by ammonium-N from organic nitrogen compounds has been eliminated by KOBr pretreatment and complete release of fixed ammonium-N in the KOBr-residue is obtained with the 5 N HF:N HCl treatment.
Comparison of KOBr-HF Method with Current HF Methods

The fixed ammonium contents of 6 soils were determined by the KOBr-HF method (Procedures A and B) as described in this section and also by the methods of Dhariwal and Stevenson (1958) and the direct and KOH methods of Bremner (1959) as described in Appendix I. The fixed ammonium values obtained by different methods are shown in Table 27, and these values calculated as percentages of the values obtained by the KOBr-HF method are presented in Table 28. These tables show that the KOBr-HF methods gave higher values for fixed ammonium in all 6 soils than the three current methods. The average values of 204 ppm. of fixed ammonium-N obtained by the KOBr-HF methods indicate that these two methods give higher estimates of fixed ammonium in soils than the three current methods and these estimates were found to be significantly higher than the average estimates for the other methods when compared using Duncan's (1955) multiple range test at the 5% probability level. Similar estimates for fixed ammonium were obtained by the Dhariwal and Stevenson method and the Bremner KOH method, 166 and 170 ppm., respectively, but these estimates were lower than that obtained by the Bremner direct method, 181 ppm. (significant at the 5% probability level using Duncan's multiple range test).
Table 27. Fixed ammonium-N values by different methods

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Method</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>9</th>
<th>10</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fixed ammonium-N (ppm. of soil)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dhariwal &amp; Stevenson</td>
<td>134</td>
<td>111</td>
<td>231</td>
<td>148</td>
<td>160</td>
<td>212</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>Bremner (KOH)</td>
<td>127</td>
<td>107</td>
<td>227</td>
<td>148</td>
<td>180</td>
<td>228</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>Bremner (direct)</td>
<td>153</td>
<td>126</td>
<td>240</td>
<td>158</td>
<td>181</td>
<td>227</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>KOBr-HF (procedure A)</td>
<td>158</td>
<td>124</td>
<td>268</td>
<td>182</td>
<td>217</td>
<td>273</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>KOBr-HF (procedure B)</td>
<td>161</td>
<td>128</td>
<td>268</td>
<td>176</td>
<td>217</td>
<td>273</td>
<td>204</td>
<td></td>
</tr>
</tbody>
</table>

Table 28. Fixed ammonium-N values by different methods calculated as percentages of the values obtained by the KOBr-HF method (procedure A)

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Method</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>9</th>
<th>10</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fixed ammonium-N (% of value by procedure A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dhariwal &amp; Stevenson</td>
<td>85</td>
<td>90</td>
<td>86</td>
<td>81</td>
<td>73</td>
<td>78</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>Bremner (KOH)</td>
<td>80</td>
<td>86</td>
<td>85</td>
<td>81</td>
<td>83</td>
<td>84</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Bremner (direct)</td>
<td>97</td>
<td>102</td>
<td>90</td>
<td>87</td>
<td>83</td>
<td>83</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>KOBr-HF (procedure B)</td>
<td>102</td>
<td>103</td>
<td>100</td>
<td>97</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

The data in Table 28 show that these three current methods released an average of 82 to 90% of the fixed ammonium present in these soils as determined by the KOBr-HF method. This incomplete release of fixed ammonium was due to inadequacies in the method of shaking, the period of HF treatment and the volume and strength of HF solution used.
(Section VI). Data presented in Section V indicate that the Dhariwal and Stevenson method and Bremner's direct method are subject to interference by organic nitrogen compounds, especially in soils with high contents of organic carbon. On the basis of these results, the KOBr-HF method appears to be more reliable than any of the current HF methods of estimating fixed ammonium.

Defects of KOBr-HF Method

The work reported leaves little doubt that the KOBr-HF method described is superior to previous methods of determining fixed ammonium in soils. It should be emphasized, however, that there is no way of proving that this KOBr-HF method or any other method, of estimating fixed ammonium in soils is accurate, and that certain basic questions concerning the reliability of HF methods have not been resolved. For example, the possibility that some ammonium-N from soil organic matter remaining in the pretreatment residues is released by HF treatment and measured as fixed ammonium-N cannot be excluded, and this point is impossible to prove by direct means. Considerable indirect evidence has been obtained in the present work to indicate that interference from this source of nitrogen is insignificant, but direct evidence on this point has not been obtained.

Another question relates to the efficiency of the HF treatments used for the release of fixed ammonium. The present work has shown that the HF treatments used in previous HF methods of estimating fixed ammonium in soils do not effect quantitative release of this ammonium, and has provided strong evidence that the HF treatment used in the
KOBr-HF method effects quantitative release of fixed ammonium. However, it is possible that soils contain some fixed ammonium which is not associated with silicate minerals, or is associated with silicate minerals which are not completely decomposed by HF and thus would not be estimated by HF procedures currently used for determining fixed ammonium.

The possibility that some of the fixed ammonium in soils may be in the form of complex ammonium phosphates (e.g., ammonium taranakite) or other types of ammonium salts which are only slightly soluble in water or KCl solution does not appear to have been considered. Haseman et al. (1950, 1951) suggested that complex ammonium phosphates may occur in soil and other workers have identified various complex ammonium phosphates as reaction products of phosphate fertilizers in soils under acid and alkaline conditions (Lindsay et al.; 1959; Lindsay and Stephenson, 1959a, b; Wada, 1959; Lindsay et al., 1962). Thus, it appears that complex ammonium phosphates can be formed under a wide range of conditions and only small amounts of these compounds are considered exchangeable by presently-accepted methods of analysis (Taylor et al., 1960, 1963; Bridger et al., 1962). From the foregoing discussion, it seems likely that complex ammonium phosphates would be classified as fixed ammonium in soils, but there is no evidence to show that any of the currently used procedures for determining fixed ammonium would include this form of ammonium.

Samples of several ammonium taranakites were obtained through the courtesy of A. W. Taylor (Division of Chemical Development, Tennessee Valley Authority, Wilson Dam, Alabama) and samples of several other metal ammonium phosphates were obtained through the courtesy of G. L.
Bridger (Research Division, W. R. Grace & Co., Washington Research Center, Clarksville, Maryland). Experiments were conducted to determine the reaction of these metal ammonium phosphates with the reagents used to determine exchangeable and fixed ammonium by several of the current procedures.¹ The data are presented in Table 29 and it appears that only relatively small amounts of these compounds are considered exchangeable by the extraction-distillation method, but widely varying amounts are considered exchangeable by the direct distillation method. The current methods for determining fixed ammonium do not measure these metal ammonium phosphates when a pretreatment is used for the removal of organic nitrogen, but both the N¹HF:N¹HCl solution of Bremner (1959) and the 5 N¹HF:N¹HCl solution proposed in the present work effect quantitative release of ammonium-N in these compounds if the pretreatment is omitted. Thus, special techniques will have to be developed for the measurement of this form of fixed ammonium in soil.

Another possibility which must be considered in the evaluation of HF methods of estimating fixed ammonium is that soils may contain very labile organic nitrogen compounds within the lattices of silicate minerals, and these compounds are not removed by the pretreatments used for removal of organic nitrogen compounds, but are released and extensively decomposed to ammonium by the treatments employed for liberation and estimation of fixed ammonium in HF methods of analysis. There is no evidence to support this possibility, but it cannot be excluded.

¹The assistance of D. Nelson in conducting these experiments is gratefully acknowledged.
Table 29. Analyses of metal ammonium phosphates

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total nitrogen (%)</th>
<th>Ammonium-N (% of total N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exchangeable</td>
<td>Fixed</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>D</td>
</tr>
<tr>
<td>Ammonium taranakite $\text{H}_6\text{(NH}_4\text{)}_3\text{Al}_5\text{(PO}_4\text{)}_8\cdot 18\text{H}_2\text{O}$</td>
<td>3.42</td>
<td></td>
</tr>
<tr>
<td>Ammonium iron phosphate $\text{H}_8\text{(NH}_4\text{)}\text{Fe}_3\text{(PO}_4\text{)}_6\cdot 6\text{H}_2\text{O}$</td>
<td>1.63</td>
<td>3</td>
</tr>
<tr>
<td>Ammonium aluminium leucophosphate $\text{(NH}_4\text{)}\text{Al}_2\text{(PO}_4\text{)}_2\text{OH} \cdot 2\text{H}_2\text{O}$</td>
<td>4.42</td>
<td></td>
</tr>
<tr>
<td>Ammonium iron leucophosphate $\text{(NH}_4\text{)}\text{Fe}_2\text{(PO}_4\text{)}_2\text{OH} \cdot 2\text{H}_2\text{O}$</td>
<td>3.57</td>
<td>2</td>
</tr>
<tr>
<td>Copper ammonium phosphate $\text{CuNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$</td>
<td>7.35</td>
<td>1</td>
</tr>
<tr>
<td>Magnesium ammonium phosphate $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$</td>
<td>5.52</td>
<td>5</td>
</tr>
<tr>
<td>Manganese ammonium phosphate $\text{MnNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$</td>
<td>7.05</td>
<td>6</td>
</tr>
<tr>
<td>Zinc ammonium phosphate $\text{ZnNH}_4\text{PO}_4$</td>
<td>7.50</td>
<td>9</td>
</tr>
</tbody>
</table>

$^a$E, extraction-distillation method; D, direct distillation method.

$^b$TE, total-N minus exchangeable ammonium-N as determined by extraction-distillation method; TD, total-N minus exchangeable ammonium-N as determined by direct distillation method; DS, method of Dhariwal & Stevenson (1958); BD, direct method of Bremner (1959); BK, KOH method of Bremner (1959); KA, KOBr-HF method (procedure A); KB, KOBr-HF method (procedure B); A, KOBr-HF method (procedure A) with KOBr pretreatment omitted.
SECTION VIII. SUMMARY AND CONCLUSIONS

The studies reported were undertaken to develop a reliable method of determining fixed ammonium in soils. A comparison of the HF methods currently favored for this determination (Table 3) showed that they gave widely divergent results and that the average fixed ammonium-N values obtained in analysis of a variety of soils by these methods decreased in the order: Rodrigues method (917 ppm.); Schachtschabel method (316 ppm.); Bremner direct method (181 ppm.); Bremner KOH method (170 ppm.); Dhariwal and Stevenson method (166 ppm.). Studies of possible sources of error in these methods showed that the major defects are that the pretreatments used to eliminate interference by organic nitrogen compounds are inefficient or lead to fixation of ammonium by soil minerals and that the procedures used for release and estimation of fixed ammonium are not quantitative or lead to formation of ammonium from organic nitrogen compounds.

The Rodrigues method was shown to be subject to extensive interference by organic nitrogen compounds in the determination of fixed ammonium (Tables 16 and 19) and also to effect incomplete release of fixed ammonium due to the short period of HF treatment (1 hour) without agitation.

The Schachtschabel method was found to be subject to considerable interference as a result of fixation of ammonium from organic nitrogen compounds during $H_2O_2$-KCl pretreatment (Tables 10 and 11) and release of ammonium from organic nitrogen compounds during alkaline distillation (Table 15).
The Bremner direct method was shown to be subject to some interference by organic nitrogen compounds (Table 3) and also to effect incomplete release of fixed ammonium due to the inadequate amount and strength of HF used (Tables 18 and 19).

The Bremner KOH method also effected incomplete release of fixed ammonium as a result of the inadequate amount and strength of HF used (Tables 18 and 19).

The Dhariwal and Stevenson method was subject to very slight interference by organic nitrogen compounds in soils with 5% or more organic carbon, but no interference in soils with low organic carbon (Table 14), due to the effectiveness of the KOH pretreatment in removing labile organic nitrogen compounds. The HF treatment used did not effect complete release of fixed ammonium because of inadequacies in the method of shaking, the period of HF treatment, and the amount and strength of HF solution used (Table 18).

A new method of determining fixed ammonium in soils was developed in which the soil sample is treated with alkaline potassium hypobromite (KOBr) solution to remove exchangeable ammonium and organic nitrogen compounds and the residue from this pretreatment is washed with 0.5 N KCl and shaken continuously with 5 N HF:N HCl (20 ml./g. of soil) for 24 hours at room temperature to decompose minerals containing fixed ammonium. The ammonium released by the HF-HCl treatment is then determined by collection and titration of the ammonium liberated by steam distillation of the soil-acid mixture with KOH.

The KOBr pretreatment used to eliminate interference by organic nitrogen compounds in this method effects almost quantitative (96 to
100% removal of organic nitrogen (Tables 6 and 7) under conditions which cause no release of fixed ammonium from vermiculite (Table 12) and involve no risk of fixation during this pretreatment (Table 9). Any ammonium formed by KOBr oxidation of soil organic matter is immediately converted to $N_2$ by the following reaction:

$$2NH_3 + 3KOB = N_2 + 3KBr + 3H_2O$$

The possibility that ammonium formed by KOBr oxidation of soil organic matter may be fixed by minerals is also precluded by the high concentration of $K^+$ in the KOBr solution used.

The experiments reported in Tables 18 and 19 provide good evidence that the HF treatment used in this method effects quantitative release of fixed ammonium and the experiments reported in Tables 14 and 23 have shown conclusively that the KOH distillation procedure used to determine the ammonium released by HF treatment is not subject to interference by organic nitrogen compounds.

The KOBr-HF method developed has high precision (Table 26) and was found to give much lower values than the procedure of Rodrigues and of Schachtschabel (Tables 3 and 28), but higher values than the procedures of Bremner direct, Bremner KOH, and Dhariwal and Stevenson (Table 28).

The possible major defects of the KOBr-HF method are also common to other methods employing HF for the determination of fixed ammonium and are listed below.

1. The possibility that some ammonium-N from organic compounds remaining in the pretreatment residues (either not removed by pretreatment or trapped in soil minerals) is released by HF treatment and
measured as fixed ammonium.

2. The possibility that soils contain some fixed ammonium which is not associated with silicate minerals, or is associated with silicate minerals which are not completely decomposed by HF and thus would not be estimated.

3. The possibility that some of the fixed ammonium in soils is in the form of complex ammonium phosphates (e.g. ammonium taranakite) and would not be measured by the KOBr-HF method (Table 29).

There is no direct evidence to support these possibilities, but they cannot be excluded.

The modified KOBr-HF method (procedure B) described in Section VII, which uses heating of the HF-KOBr residue mixture for 30 minutes at 100°C, for the release of fixed ammonium, has the advantage that the treatment with HF requires only 30 minutes instead of 24 hours. This modification may have advantages for routine analysis of soils, however, it may not be satisfactory for soils containing large amounts of organic matter, and it is recommended that procedure A be used for analysis of organic soils. A comparison (Table 21) using six soils with organic carbon contents ranging from 0.79 to 10.2% showed that the average fixed ammonium-N values by procedures A and B were identical (204 ppm.).


Gieseking, J. E. 1949. The clay minerals in soils. Advances in Agronomy 1: 159-204.


ACKNOWLEDGMENTS

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The author is also indebted to Dr. D. V. Huntsberger for his helpful suggestions and advice.
APPENDIX I

Description of Current HF Methods

Rodrigues (1954)

Place 2 g. of soil in a 100-ml. polypropylene centrifuge tube, add
2 ml. of 50% (v/v) H₂SO₄ and 8 ml. of 40% (w/w) HF, and allow the mixture
to stand. After 1 hour, add 30 ml. of water, stopper the tube, and shake
the tube on a mechanical shaker for 5 minutes. Then centrifuge the tube
for 30 minutes and decant the supernatant into a polyethylene storage
container. Place a 5-ml. aliquot of the supernatant in a 30-ml. Kjeldahl
flask, add 1 ml. of concentrated H₂SO₄, and heat the mixture on a
Kjeldahl digestion rack for about 45 minutes to drive off HF. Allow the
mixture to cool, and transfer it through a long-stemmed polyethylene
funnel to a 100-ml. distillation flask which contains 7 ml. of 10 N NaOH
and is marked to indicate a volume of 35 ml. Perform this transfer with
the end of the stem of the funnel below the surface of the NaOH solution.
Rinse the Kjeldahl flask and funnel with water until the level of the
liquid in the distillation flask reaches the 35-ml. mark. Stopper the
flask, swirl it to mix the contents, and after allowing it to stand for
a few minutes, attach it to the steam-distillation apparatus described
in Section VII (Figure 2). Steam distill the mixture and collect the
distillate in a 100-ml. Erlenmeyer flask which contains 10 ml. of boric
acid-indicator solution and is marked to indicate a volume of 70 ml.
When the distillate reaches the 70-ml. mark on the receiver flask, stop
the distillation, rinse the end of the condenser, and determine ammonium-N in the distillate by titration with 0.005 N H\textsubscript{2}SO\textsubscript{4}. To calculate fixed ammonium-N, subtract the amount of exchangeable ammonium-N in the sample as determined by analysis of a separate sample using the extraction-distillation method described in Section III.

In the description of his method, Rodrigues did not specify the concentration of the HF solution used, the amount of water employed for dilution of the HF-soil mixture, the period of heating the extract with H\textsubscript{2}SO\textsubscript{4} to drive off HF, or the base used for distillation. The procedure described here is similar to that adopted by Bremner (1959).

Dhariwal and Stevenson (1958)

Place 1 g. of soil in a 200-ml. tall-form beaker, add 25 ml. of N KOH, and autoclave the mixture for 8 hours at 20 pounds pressure and 120°C. Allow the beaker to cool, transfer the contents with 0.5 N KCl to a 100-ml. polypropylene centrifuge tube, centrifuge, and decant the supernatant. Then add 20 ml. of 0.5 N KCl, fit the neck of the tube with a rubber stopper, and, after shaking the tube manually for a few seconds, centrifuge it and decant the supernatant. After performing this washing operation 3 times, add 7.5 ml. of 5 N HF:0.75 N HCl:0.6 N H\textsubscript{2}SO\textsubscript{4}, stopper the centrifuge tube, and allow the mixture to stand at room temperature for 16 hours with occasional shaking (shake the mixture for 5 minutes at 3-hour intervals). Then transfer the soil-acid mixture through a long-stemmed polyethylene funnel to a 100-ml. distillation flask which contains 6 ml. of 10 N NaOH and is marked to indicate a volume of 35 ml. Perform this
transfer with the end of the stem of the funnel below the surface of the NaOH solution. Rinse the centrifuge tube and funnel with water until the level of liquid in the distillation flask reaches the 35-ml. mark. Stopper the flask, swirl it to mix the contents, and, after allowing the flask to stand for several minutes, attach it to the steam-distillation apparatus described in Section VII (Figure 2). Then determine the amount of ammonium-N liberated by steam distillation using the technique adopted in performance of the Rodrigues method.

Bremner (1959)-direct method

Place 1 g. of soil in a 100-ml. polypropylene centrifuge tube and add 40 ml. of N HF:N HCl and 1 drop of octyl alcohol. Fit the neck of the tube with a rubber stopper, and shake the tube on a mechanical shaker for 24 hours at room temperature. Then centrifuge the tube for 5 minutes and filter the supernatant (Whatman no. 40 filter paper). Place a 15-ml. sample of the filtrate in a 100-ml. distillation flask and neutralize the acid in the sample by cautious addition of 2 N NaOH using phenolphthalein as indicator. After neutralization, add 10 ml. of sodium borate buffer (pH 8.8), stopper the flask, swirl it to mix the contents, and allow it to stand for several minutes. Then attach the flask to the steam-distillation apparatus described in Section VII (Figure 2) and determine the amount of ammonium-N liberated by steam distillation using the technique adopted in performance of the Rodrigues method. To calculate fixed ammonium-N, subtract the amount of exchangeable ammonium-N in the sample as determined by analysis of a separate sample using the extraction-distillation method described in Section III.
**Bremner (1959)-KOH method**

Place 1 g. of soil in a 200-ml. tall-form beaker, add 40 ml. of 0.5 N KOH, and boil the mixture gently on a hotplate until its volume has been reduced to about 20 ml. Transfer the cooled mixture with N KCl to a 100-ml. polypropylene centrifuge tube, centrifuge, and decant the supernatant. Add 20 ml. of N KCl, fit the neck of the tube with a rubber stopper, and, after shaking the tube manually for a few seconds, centrifuge and decant the supernatant. Repeat this washing operation and then perform an additional wash using 0.5 N KCl instead of N KCl. Then place the tube in a vacuum desiccator containing KOH, and, when the soil residue is dry, add 40 ml. of N HF:N HCl and 1 drop of octyl alcohol. Fit the neck of the tube with a rubber stopper, and shake the tube on a mechanical shaker for 24 hours at room temperature. Centrifuge the mixture, filter the supernatant (Whatman no. 40 filter paper), and determine ammonium-N in an aliquot of the filtrate as described in the previous section.

**Schachtschabel (1961b)**

Place 10 g. of soil in a 600-ml. beaker, add 5 ml. of 10% (1.3 N) KCl and 25 ml. of 30% H₂O₂, cover the mouth of the beaker with a watch glass, and allow the beaker to stand overnight at room temperature. Then place the covered beaker in a water bath (100°C.), and, after 1 hour, add an additional 25 ml. of 30% H₂O₂ and heat the beaker in the water bath for 2 hours. Allow the mixture to cool and settle, and decant the supernatant through a sintered glass funnel fitted with a Whatman no. 50 filter paper. Add 300 ml. of 0.05 N HCl to the residue in the beaker, and, after stirring, allow the mixture to settle and decant the supernatant through
the sintered glass funnel. Repeat this operation using 300 ml. of 0.05 N HCl and then 300 ml. of water, and transfer the residue to the sintered glass funnel with 95% ethanol and wash it in the funnel with 300 ml. of 95% ethanol. Dry the washed residue overnight in an oven at 50°C., weigh it, and grind it to pass a 100-mesh sieve.

Place 1g. of the residue from the H₂O₂ pretreatment in a 25-ml. platinum crucible, and add 2 ml. of concentrated H₂SO₄. After 1 hour, heat the crucible on a sand bath until the appearance of white fumes. Allow the crucible to cool, add 2 ml. of water, and, after about 15 minutes, add 3 ml. of 40% (w/w) HF and heat the crucible on the sand bath until the mixture has boiled for about 1 hour. Then add an additional 3 ml. of 40% HF, stir the mixture with a platinum wire, and, after heating the mixture for about 40 minutes, add another 3 ml. of 40% HF and again stir the mixture. Evaporate the mixture on the sand bath until the appearance of white fumes. Allow the crucible to cool and place it in a 200-ml. beaker. Add about 30 ml. of water, and heat the beaker on the sand bath for about 30 minutes to loosen the residue from the sides of the crucible. Then transfer the contents of the crucible to the beaker with water and subsequently transfer the soil-acid mixture in the beaker through a long-stemmed polyethylene funnel to a 250-ml. distillation flask which contains 25 ml. of 10 N NaOH and is marked to indicate a volume of 90 ml. Perform this transfer with the end of the stem of the funnel below the surface of the NaOH solution. Rinse the beaker and funnel with water until the level of the liquid in the flask reaches the 90-ml. mark. Then stopper the flask, swirl it to mix the contents, and, after allowing the flask to stand for several minutes, attach it to the steam-
distillation apparatus described in Section VII (Figure 2). Then determine the amount of ammonium-N liberated by steam distillation using the technique adopted in performance of the Rodrigues method, but collect the distillate in a 250-ml. Erlenmeyer flask which contains 10 ml. of boric acid-indicator solution and is marked to indicate a volume of 120 ml. Stop the distillation when the distillate reaches this mark. To calculate fixed ammonium-N, determine the amount of carbon in the peroxide-treated soil by the modified Allison (1960) method described in Section III., and subtract 3 mg. of ammonium-N for every 100 mg. of carbon in the peroxide-treated soil.
APPENDIX II

Description of Pretreatments

\[ \text{N KOH} \]

Place 1.0 g. of soil (or 0.5 g. of vermiculite) in a 200-ml. tall-form beaker, add 25 ml. of \( \text{N KOH} \), and autoclave the mixture for 8 hours at 20 pounds pressure and 120°C. Transfer the cooled mixture with 0.5 N KCl to a 100-ml. polypropylene centrifuge tube and wash the pretreatment residue as described below.

\[ \text{0.5 N KOH} \]

Place 1.0 g. of soil (or 0.5 g. of vermiculite) in a 200-ml. tall-form beaker, add 40 ml. of 0.5 \( \text{N KOH} \) and boil the mixture gently on a hotplate until its volume has been reduced to about 20 ml. Transfer the cooled mixture with 0.5 N KCl to a 100-ml. polypropylene centrifuge tube and wash the pretreatment residue as described below.

\[ \text{H}_2\text{O}_2-\text{KCl} \]

Place 1.0 g. of soil (or 0.5 g. of vermiculite) in a 100-ml. tall-form beaker and add 0.5 ml. of 1.3 N KCl and 2.5 ml. of 30% \( \text{H}_2\text{O}_2 \). Cover the mouth of the beaker with a watch glass and allow the mixture to stand overnight at room temperature. Then place the covered beaker in an oven at 100°C. for 1 hour, and, during this period of heating, add water at intervals to keep level of liquid in the beaker approximately constant. Then add an additional 2.5 ml. of 30% \( \text{H}_2\text{O}_2 \) and heat the mixture at 100°C. for 2 hours, adding water at intervals as in the first period of heating. Allow the beaker to cool, transfer the contents with 0.5 N KCl to a 100-ml. polypropylene centrifuge tube, and wash the pretreatment residue as
described below.

**KOBr**

Place 1.0 g. of soil (or 0.5 g. of vermiculite) in a 200-ml. tall-form beaker, and add 20 ml. of KOBr solution prepared by addition of 6 ml. of bromine to 200 ml. of ca. 2 N KOH. [Add the bromine slowly (ca. 0.5 ml./min.) and with constant stirring, and cool the KOH solution in an ice bath during this addition]. Swirl the beaker to mix the soil and hypobromite and then allow the beaker to stand at room temperature with its mouth covered by a watch glass. After 2 hours, add 60 ml. of water, and heat the covered beaker on an electric hotplate until the soil-hypobromite mixture has boiled vigorously for 5 minutes. Allow the mixture to cool and settle in the covered beaker (preferably overnight), and decant and discard the clear supernatant. Transfer the residue with 0.5 N KCl to a 100-ml. polypropylene centrifuge tube and wash the pretreatment residue as described below.

**2 N KCl**

Place 1.0 g. of soil (or 0.5 g. of vermiculite) in a 100-ml. polypropylene centrifuge tube and add 15 ml. of 2 N KCl. Fit the neck of the tube with a rubber stopper and shake the tube for 1 hour on a mechanical shaker. Then centrifuge the mixture, decant the clear supernatant, and wash the pretreatment residue as described below.

**Washing**

After transfer of the pretreatment residue to a 100-ml. polypropylene centrifuge tube, add sufficient 0.5 N KCl to bring the total volume to 80 ml. Stopper the tube, shake it manually for a few seconds, and centrifuge it for 10 minutes. Decant the clear supernatant, add 80 ml. of
0.5 N KCl, and shake and centrifuge the tube as in the previous operation. Decant the supernatant.
APPENDIX III

Determination of Ammonium Released by HF Treatment

After completion of the treatment with HF solution, transfer the contents of the centrifuge tube through a long-stemmed polyethylene funnel to a 250-ml. distillation flask which is marked to indicate a volume of 60 ml. and contains 1.5 ml. of 10 N KOH for every 12 me of N acid used in the HF treatment. Perform this transfer with the end of the stem of the funnel below the surface of the KOH solution. Wash the centrifuge tube and funnel with water until the level of the liquid in the flask reaches the 60-ml. mark. Then stopper the flask, swirl it to mix the contents, and allow it to stand for a few minutes. During this period of standing, add 10 ml. of boric acid-indicator solution to a 250-ml. Erlenmeyer flask which is marked to indicate a volume of 100 ml., and place the flask under the condenser of the steam-distillation apparatus so that the end of the condenser is about 4 cm. above the surface of the boric acid. Attach the distillation flask to the steam-distillation apparatus described in Section VII (Figure 2), and immediately commence distillation by closing the stopcock on the steam by-pass tube of the distillation apparatus. When the distillate reaches the 100-ml. mark on the receiver flask, stop the distillation by opening the stopcock on the steam by-pass tube (period of distillation, ca. 12 minutes). Then rinse the end of the condenser, and determine ammonium-N in the distillate by titration with 0.005 N sulfuric acid from a micro-burette (1 ml. 0.005 N H₂SO₄ = 70 μg. NH₄-N).