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THE FIXATION OF AMMONIA AND
NITRATE BY ORGANIC MATERIALS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Sadao Shoji

1958

THE FIXATION OF AMMONIA AND
NITRATE BY ORGANIC MATERIALS

By

SADAO SHOJI

AN ABSTRACT

Submitted to the College of Agriculture
Michigan State University of Agriculture and
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the requirements for the degree of

MASTER OF SCIENCE

Department of Soil Science

1958

Approved

Max M. Mortland
A. R. Wolcott

ABSTRACT

Non-biological fixation of nitrogen by soils and lignin was studied in the laboratory in relation to oxidation and associated changes in cation exchange capacity, methoxyl content and methylating capacity.

No nitrate was fixed at any pH by any of the materials. Extensive oxidative fixation of ammonia by muck soils and lignin was influenced by pH, concentration of ammonia or ammonium ion and by the nature of associated cations.

Methylation of organic materials with dimethyl sulfate blocked ammonia fixation and decreased cation exchange capacity about one-third.

Oxidation with NaOH resulted in a seven-fold increase in cation exchange capacity of lignin and a 10 to 15% increase in exchange capacity of muck soils. Oxidation with NH_4OH resulted in a two-fold increase in exchange capacity of lignin but a 10 to 15% decrease in that of muck. It appeared that oxidative ammonia fixation involved two opposing processes with reference to its effect on cation exchange capacity: Oxidative processes increased exchange capacity, as in the case of NaOH, whereas associated reactions with ammonia resulted in disappearance of active exchange sites formed during oxidation.

Acid leaching and MgO distillation removed similar quantities of fixed nitrogen. A smaller proportion of fixed nitrogen was removed from lignin than in the case of the muck

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Ultraviolet absorbance spectra of lignin showed a well-defined minimum at 262 $m\mu$ and a sharp maximum at 283 $m\mu$. Oxidation with NaOH shifted these two points in the direction of longer wavelengths, but did not materially alter the shape of the curve. Oxidative ammonia fixation altered the shape of the absorbance curves for lignin by largely obscuring the minimum and maximum peaks in this range, producing curves with gentle slopes, similar to the spectra for muck materials.

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INTRODUCTION

Classical studies with organic matter in plant residues, litter and humus have shown that ammonia is extensively fixed in chemically and biologically resistant forms by the lignin or lignin-like constituents of these materials. Such fixation may have a significant influence on the availability of fertilizer nitrogen when high concentrations of nitrogen are introduced into localized zones of the soil by band placement techniques and by applicators used to apply ammonia in gaseous form or in water solutions to soils. This may be particularly true in soils which are high in organic matter. Experience in fertility studies on organic soils in Michigan has shown that crops which respond to spring applications or top dressings of ammonia fertilizers frequently fail to respond to fall applications.

The present studies were carried out to determine the extent of fixation of nitrogen by lignin and muck soil, and to study some of the conditions which might influence it. Also, it was of interest to determine something of the nature of the mechanisms whereby fixation occurs and the changes in cation exchange properties which result.

REVIEW OF LITERATURE

Soil organic matter has been the subject of many investigations. A great difficulty in these studies has been that humic materials can not be precisely separated from the unhumified organic matter. In other words, the soil organic matter consists of a whole series of products ranging from undecomposed plant and animal tissues to so-called "humified", stable, amorphous, black material. Some of these organic materials are intimately associated with the clay minerals in complexes from which they can be released with difficulty by extracting procedures which probably alter their essential chemistry. Bremner (3) has summarized the present status of knowledge about soil organic matter by saying that although no great progress has been made since Waksman's comprehensive review (24) of the subject in 1938, much useful information has been obtained and several points have been clarified.

Marshall (11) reviewed the literature on physico-chemical studies of soil organic matter and concluded that knowledge of the physical chemistry of soil humic matter has advanced very little since the classical work of Odén published in 1912. Physico-chemical work on humus since 1920 has largely centered around four topics: Characterization by pH titration curves, chemical and physical properties under saturation by various cations, and utilization of the scanty X-ray evidence available to throw light upon molecular

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structure.

Many investigators have worked on the structure and molecular weight of humic acid. Waksman (24) found that the chemistry of various "humic acids" depended upon the method used in their separation, especially upon the nature and concentration of the alkali and acid, and upon the length of treatment. It is not surprising, therefore, that no exact comparison could be made between the results obtained by different investigators.

Odén's (24) physico-chemical work on humus suggested the formula $C_{64}H_{52}O_{32}$ (mol. wt. = 1332) for "humus acids" containing three to four $-COOH$ groups. Fuchs (24) showed that the molecular weight of humic acid obtained from peat was 1300 to 1400 and it contained three to four $-COOH$ groups, three to four $-OH$ groups, one methoxyl group and one carbonyl group. The $-OH$ groups were found to be active in methylation and base-binding. Broadbent and Bradford (6) showed that the probable cation-exchange groupings in soil organic matter were carboxyl groups and phenolic hydroxyl or enolic hydroxyl groups.

The study of the processes of humus formation is one of the most interesting problems. The restricted knowledge of humic matter constitution makes the study difficult. The humic matter in the soil has been considered to be derived from two chief sources: (a) lignins in plant residues and (b) products of microbial metabolism. Waksman and Iyer (25) proposed the ligno-protein complex theory. Though there is

no direct proof of this theory, it is supported indirectly by the fact that protein is resistant to microbial decomposition in the presence of lignin (5).

From Bremner's (3) fractionations of organic nitrogen, it appears that a third to two thirds of soil organic nitrogen is present as protein derivatives. Some of the remainder is thought to exist as heterocyclic nitrogen compounds. Mattson and Kouttler-Anderson (12, 13, 14) presented the ligno-ammonium complex theory, suggesting that some of the stable nitrogen compounds in the soil might be produced by the reaction of oxidized lignin and ammonia (or possibly aromatic amines) at the sites of phenolic hydroxyl groups to form amidophenols. Upon oxidation and condensation, these would form polymers containing ring nitrogen.

The change of lignin to humic matter seems to involve two processes: oxidation and enrichment with nitrogen. The oxidation results in an increase of carboxyl groups (16) and probably phenolic groups. This oxidation takes place spontaneously in the presence of air in an alkaline medium (4, 9). If ammonia is present during oxidation, some of the ammonia is incorporated chemically with the lignin and appears in the oxidized products (9, 12). The mechanisms of these reactions are not known.

Some of the early studies on ammonia fixation seem to have been made in connection with coal chemistry. Schrader (18) conducted experiments on the auto-oxidation of lignin

and natural humins. He found that lignin was fairly rapidly converted into humic acid in an NaOH medium containing NH_3 , oxygen being absorbed from the air concurrently with ammonia fixation. Very little NH_3 was absorbed in the same medium when exposed to an atmosphere of N_2 gas.

Kappen et al (10, 23) worked on problems relating to the potential value of brown coal as a fertilizer. They observed that treatment of coal with gaseous or liquid ammonia caused some combination of nitrogen in unavailable forms.

Springer (21) performed experiments on the influence of ammoniation on the organic material in high moor peats. He concluded that by ammoniation the humus forming complexes and organic pre-humus materials could be converted more or less completely into humic materials. The extent of ammoniation was greater at higher temperature and pressures, and was dependent upon other reaction conditions..

Sohn and Peech (20) reported that heating an ammoniated organic matter reduced the ammonia-retention but increased the ammonia-fixing capacity.

Problems of oxidation and nitrogen enrichment in soil organic matter were approached by Junker (9). He checked the absorption of ammonia by dioxane spruce lignin and a Scholler lignin, using Bancroft's phase rule apparatus and obtained curves showing three flat portions in the sorption isotherm. These plateaus occurred at 0.72, 1.44 and 2.76 milliequivalents NH_3 per gram of sample. The curve indicated a superposition

of absorbed and chemically combined ammonia on the lignin. He suggested that the combination of ammonia with lignin took place in three different stages, two of which might correspond to the combination of ammonia with phenolic hydroxyl groups, the third might indicate the combination of ammonia with aldehyde groups or alcoholic hydroxyl groups.

He also carried out experiments involving the absorption of oxygen increased with increasing NaOH concentration of the lignin sol. Equivalent concentrations of NaOH, KOH and $\text{Ba}(\text{OH})_2$ gave equal oxygen absorption and the absorption in alkaline earth solutions decreased in the order-- $\text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2 > \text{Mg}(\text{OH})_2$. Lignin in distilled water also absorbed oxygen. The absorption of oxygen in alkaline solutions was decreased in the presence of $\text{Mn}(\text{OH})_3$ or $\text{Fe}(\text{OH})_3$. Oxidized spruce lignin was more easily dispersed and had better base-binding properties than electro-dialyzed lignin.

Mattson and Kouttler-Andersson (12) investigated ammonia fixation by different forms of litter and humus, by water-soluble organic matter and lignin fractions, and by a series of organic compounds of known constitution. The ammonia fixation was greatest under conditions of simultaneous auto-oxidation. Very small amounts of ammonia were fixed under anaerobic conditions.

The water-soluble and lignin fractions fixed more ammonia per unit weight of organic matter than the whole sample. The lower the base status of the litter and humus, the

greater was the absorption of oxygen and the fixation of ammonia. The ammoniated complex was very stable to acids, the nitrogen being largely carried over in the lignin fraction obtained by 72% sulfuric acid treatment. Strong alkali removed nearly half of the fixed ammonia.

They suggested that the stable nitrogen compounds in soil might be produced by the reaction of ammonia, or possibly aromatic amines, with oxidized lignin at the sites of phenolic hydroxyl groups to form amidophenols. These would form polymers containing ring nitrogen when oxidized and condensed.

More recently, studying peat formation, the same authors (13, 14) found that while auto-oxidation increased the base-binding capacity of peat, ammonia fixation depressed this capacity. Finally they proposed that the resistant residue of organic decomposition and transformation which forms the permanent humus is a ligno-nitrogen complex.

Bennet's (1) methylation studies with lignin seem to support Mattson's theory. Compared with the amount of ammonia fixed during oxidation, the increase of methoxyl content in methylated and oxidized lignin was disproportionately low.

In the process of change of lignin to humic matter, methoxyl groups decrease and cation exchange capacity increases, possibly because of an increase in carboxyl groups (16, 23).

Using conifer seedlings, Themnitz (22) studied the availability of nitrogen fixed by raw humus. He showed that

the nitrogen fraction was firmly held in ammoniated humus, possibly in a heterocyclic bond. It was not distilled off in the presence of MgO and was poorly available to the seedlings.

Spectrographic absorption studies over the ultra-violet and visible ranges show differences between humic materials and lignin. Absorption curves for lignin have much steeper slopes than those for humic matter. Maximum absorbance for lignin occurs at about 280 mu and the minimum at about 260 mu (2, 8, 17, 19). Spectra for humic materials show a generally declining absorbance with increasing wavelengths, with no distinct maxima or minima.

OBJECTIVES

The objectives of the present study were to study the non-biological fixation of nitrogen by organic soils and by lignin. It was desired to study fixation as affected by nitrogen source and conditions of reaction, including pH, concentration and associated bases. The relation of fixation to cation exchange capacity was of interest. By the use of methylating procedures it was hoped that some information might be derived relative to the mechanisms whereby nitrogen is fixed.

MATERIALS AND METHODS

Experimental Materials

Experimental materials used in this experiment included the following: commercial lignin*, Sims silt loam (humic glei soil), and samples of Houghton muck soil, taken from a virgin area, a reclaimed area unfertilized for fifteen years and a reclaimed area which had been heavily fertilized for fifteen years. These materials were air-dried and passed through a 2 mm. sieve before being subjected to the various laboratory treatments.

Experimental Treatments

Fixation of nitrate nitrogen

Fifty ml. of 1N NaNO_3 and a drop of toluene were placed in a 500 ml. Erlenmeyer flask containing 2 g. material (10 g. for Sims silt loam). The pH in different flasks was adjusted with NaOH and/or HNO_3 to 2, 4, 7, and 9. The flasks were closed with two-hole rubber stoppers to allow for aeration. They were then shaken continuously for three days. The contents of the flasks were then neutralized with dilute HCl or NaOH. The precipitate in each flask was filtered off and was washed with neutral 0.5N KCl to remove free and exchangeable NH_4^+ and NO_3^- . The total nitrogen of the precipitate was determined by the Kjeldahl method using Devarda's

*"Indulin A", a product of the West Virginia Pulp and Paper Co.

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alloy to reduce any NO_3 that may have been fixed.

A similar quantity of the original material was taken, neutralized with dilute HCl or NaOH , and washed with neutral 0.5 N KCl as the above. Total nitrogen was determined by the same procedure. The difference between these two total nitrogen determinations was expressed as me. per 100 g. of material.

Fixation of ammonia nitrogen

A 2-g. sample of the original material (10 g. for Sims silt loam) was placed in a 500 ml. Erlenmeyer flask. Fifty ml. of NH_4Cl or 12% NH_4OH solution and one drop of toluene were added to the flask. In the case of the NH_4Cl solution, the pH of the contents was adjusted to a given value using NH_4OH , HCl , NaOH or $\text{Ca}(\text{OH})_2$, according to the purpose of the experiment. The flask was fitted with a two-hole rubber stopper through which air could freely enter. The flask was shaken continuously for three days. Then the contents were neutralized with dilute HCl or NaOH , the precipitate was filtered off and was washed with neutral 0.5N KCl until ammonia was no longer detected in the filtrate. The total nitrogen of the residue was determined by the Kjeldahl procedure. This nitrogen determination was considered to contain both the nitrogen in the original material (N_0) and the ammonia fixed during treatment (N_f).

A separate 2-g. aliquot of the original material was neutralized, washed as the above and the total nitrogen of

the residue (N_o) was determined. "Fixed nitrogen" was determined as the difference between total nitrogen in the treated sample ($N_o + N_f$) and total nitrogen in the original material (N_o). This was converted to me. per 100 g.

Oxidation and methylation studies

Eight treatments were designed to study the relationships between ammonia fixation, methylating capacity and cation exchange of lignin, fertilized reclaimed muck, and virgin muck. These treatments were as follows:

- (a) Original material.
- (b) Original material subjected to oxidative ammonia fixation as previously described, using 12% NH_4OH and a three-day shaking period.
- (c) Original material subjected to methylation, as follows:

Two grams of the original material were allowed to react for 24 hours with 1 ml. of dimethyl sulfate in the presence of 50 ml. of 0.2N KOH in an atmosphere of nitrogen. After the treatment, the suspension was acidified with dilute acid. The precipitate was centrifuged and washed with distilled water. This treatment was repeated five times on each sample to assure complete methylation.

- (d) Ammonia-treated material (b) subjected to

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methylation as in (c).

- (e) Methylated material (c) subjected to ammonia treatment as in (b).
- (f) Original material subjected to oxidation with NaOH as follows:

Fifty ml. of 0.5N NaOH were placed in a 500 ml. Erlenmeyer flask containing 2 g. of the material. The flask was fitted with a two-hole rubber stopper and shaken continuously for three days. Then, the suspension was neutralized with dilute HCl and the precipitate was washed with distilled water.

- (g) Sodium hydroxide-treated material (f) subjected to methylation as in (c).
- (h) Methylated material (c) subjected to NaOH-oxidation as in (f).

Chemical and Physical Determinations

The following chemical and physical determinations were made on the original materials and/or the materials after various treatments:

1. The pH of original materials was determined using a glass electrode and a 1:1 soil to water ratio.
2. Ash content of original materials was determined by ignition at 700°C.
3. Cation exchange capacity of original and treated materials was determined using the barium acetate

method described by Wilson and Staker (27).

4. Total nitrogen of original and treated materials was determined by the Kjeldahl method, using a mixture of CuSO_4 and K_2SO_4 (1:9) as the catalyst. Devarda's alloy was used when it was desired to include NO_3^- .
5. The stability of fixed nitrogen was determined by its resistance to removal by leaching with 0.1N HCl or by distillation of the KCl-extracted material in the presence of MgO. The procedure used was as follows:

Using 12% NH_4OH , a 2-g. sample of the original material was subjected to oxidative ammoniation as in the (b) treatment described above. This treated material was leached with successive portions of 0.1N HCl to a final volume of about 1000 ml. The total nitrogen of the residual material was determined. Another treated sample was leached with neutral 0.5N KCl until ammonia disappeared in the filtrate. The total nitrogen of the residual material was determined. The difference between these two total nitrogen determinations was expressed as "nitrogen removed by acid treatment".

A 2-g. sample of material subjected to oxidative ammonia fixation as above and another untreated sample were leached with neutral 0.5N KCl

until exchangeable and free ammonia were removed. Then the nitrogen released during distillation in the presence of excess MgO for 45 minutes was determined. The difference between the nitrogen removed by MgO from treated and untreated materials was calculated as me. per 100 g. of material.

6. The methoxyl content of original and treated materials was determined, using a semi-micro alkoxyl apparatus, by a modification of the official AOAC procedure (15) suggested by the chemistry section of the West Virginia Pulp and Paper Co. (26). The apparatus used is shown in Figure 1.

The trap of the apparatus was filled with distilled water. A 10% solution of potassium acetate in glacial acetic acid, to which had been added 6 drops of bromine, was introduced into the absorption tubes. Six ml. of this reagent was poured in the first tube and 4 ml. in the second. About 20 to 30 mg. of sample material and one drop of Hg were placed in the digestion flask. Melted phenol (2.5 ml.) and 5 ml. of HI were added. The digestion flask was connected to the absorption tubes. Nitrogen gas was passed through the apparatus from the side arm of the flask at a uniform rate. The liquid in the digestion flask was boiled at such a rate that vapors of boiling

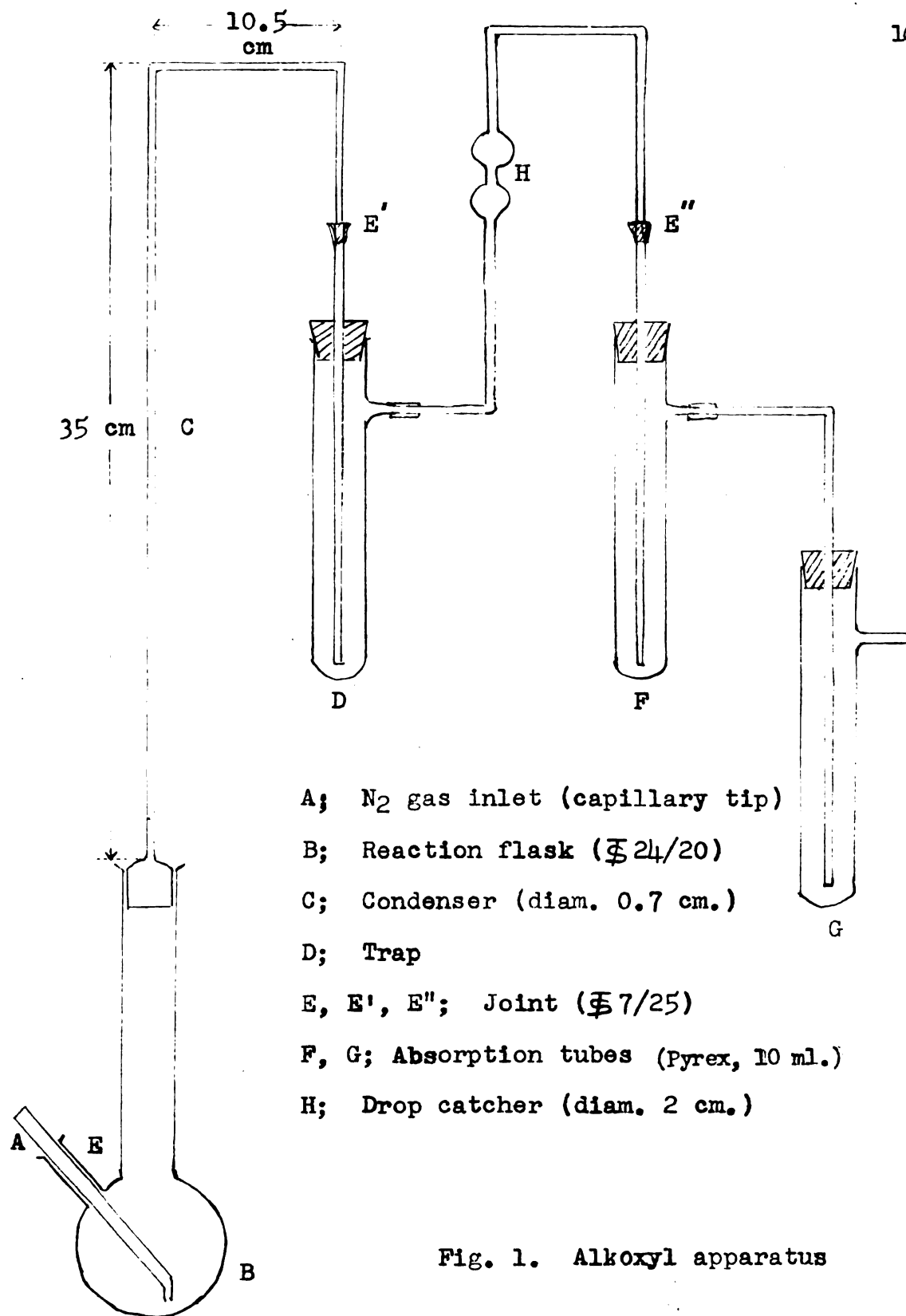


Fig. 1. Alkoxyl apparatus

liquid rose about half way up the condenser.

Boiling was continued for 60 minutes. The flask was disconnected and the absorption tube was removed. The contents of the tubes were washed into 125 ml. Erlenmeyer flasks containing 5 ml. of 25% aqueous sodium acetate solution. The volume was adjusted to about 50 ml., and formic acid was added dropwise until excess Br_2 was destroyed.

Any Br_2 vapors were removed by blowing air over the liquid. Then 0.5 g. of KI and 5 ml. of 10% H_2SO_4 were added. The solution was swirled to dissolve KI and to mix the contents. Liberated I_2 was titrated with $\frac{N}{40}$ $\text{Na}_2\text{S}_2\text{O}_3$ solution. A blank determination was run, using all reagents, without the sample. The percent methoxyl content was calculated as follows:

$$\frac{(\text{ml. of } \text{Na}_2\text{S}_2\text{O}_3 \text{ in determination} - \text{ml. in blank}) \times \frac{1}{40} \times 5.17 \times 100}{\text{sample weight (mg)}}$$

Ultraviolet Absorption

Ultraviolet absorption spectra were developed for the following:

- (a) Original lignin and fertilized muck.
- (b) Lignin and fertilized muck after oxidative ammonia fixation.
- (c) Lignin and muck after methylation.
- (d) Lignin and muck after NaOH oxidation.

A Beckman DK-2 recording spectrophotometer was used. The materials were suspended in 0.1N NaOH in suitable dilutions for effective observation over the range of wavelengths from 240 to 350 mμ.

RESULTS

Properties of Materials

Table 1 shows the properties of materials used for this research. Sims silt loam and virgin muck both had a pH of 6.2. Lignin was the lowest at pH 3.3. The Sims silt loam contained the highest percentage ash, 94.4% and lignin had the lowest, 9.2%. All the mucks contained less than 20% ash. The nitrogen in the Sims soil and in lignin was much less than that in muck. Among mucks, nitrogen content decreased in the order: Fertilized > virgin > unfertilized. The greatest cation exchange capacity was found in the muck: A maximum of 164.4 me. per 100 g. was found in the nonfertilized reclaimed muck, 161.0 me. in the fertilized, and 142.1 me. in the virgin muck. On the other hand, the cation exchange capacities of the Sims soil and of lignin were 22.3 and 32.9 me. per 100 g., respectively.

Fixation of Nitrate Nitrogen

The results of experiments conducted to determine the effect of pH on the fixation of nitrate nitrogen by lignin and soil materials are presented in Table 2. No nitrate nitrogen was fixed by any of the materials. Negative fixation was obtained for virgin muck, and the net loss of nitrogen was slightly greater in both acid and alkaline solutions than at neutrality. In any case, the amounts of nitrogen lost were less than 4 me. per 100 g.

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TABLE 1.
PROPERTIES OF THE ORIGINAL EXPERIMENTAL MATERIALS

| Materials | pH | Ash content % | Total N % | CEC me./100g. |
|--------------------|-----|------------------|--------------|------------------|
| Sims silt loam | 6.2 | 94.4 | 0.20 | 22.3 |
| Virgin muck | 6.2 | 17.9 | 3.27 | 142.1 |
| Nonfertilized muck | 5.4 | 19.5 | 3.07 | 164.4 |
| Fertilized muck | 5.7 | 18.7 | 3.32 | 161.2 |
| Lignin | 3.3 | 9.2 | 0.17 | 32.9 |

TABLE 2.
FIXATION OF NITROGEN FROM IN NaNO_3 SOLUTIONS
ADJUSTED TO VARIOUS pH'S

| pH* | Sims silt loam me./100g. | Virgin muck me./100g. | Lignin me./100g. |
|-----|-----------------------------|--------------------------|---------------------|
| 2 | - | -2.2 | - |
| 4 | - | -2.0 | - |
| 7 | - | -2.0 | - |
| 9 | - | -3.8 | - |

*) pH was adjusted with NaOH and/or HNO_3 .

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Fixation of Ammonia Nitrogen and Factors Affecting It

Influence of pH on oxidative ammonia fixation

For this experiment, IN NH_4Cl was used and pH was adjusted to 4, 7, 9, and 10.5 with NH_4OH , and/or HCl . The results are shown in Table 3 and Figure 2. Sims silt loam fixed a small amount of ammonia from acid solution but none on the alkaline side of neutrality. All the organic materials fixed much more nitrogen on the alkaline side than at neutrality or below. In the case of lignin, the amount of fixed nitrogen was 100.4 me. per 100g. at pH 10.5. This was the highest for any material. Though fertilized muck fixed slightly more ammonia than virgin muck, both fixed less than 30 me. per 100 g. When the materials were shaken in 12% NH_4OH without any adjustment of pH, ammonia fixation was increased to 32.4, 35.3 and 168.0 me. per 100 g. for virgin and fertilized mucks and lignin, respectively.

Influence of bases and concentration of NH_4Cl on ammonia fixation at pH 7.5

In Table 4 and Figures 3, 4 and 5 are presented data showing the influence of bases used for pH adjustment and concentration of NH_4Cl solution on the oxidative fixation of ammonia. It was found that the amount of ammonia fixed increased with increasing concentration of NH_4Cl with all materials, whether NaOH or $\text{Ca}(\text{OH})_2$ were used to adjust the solution to pH 7.5. However, at any given pH, more ammonia

TABLE 3.
INFLUENCE OF pH ON OXIDATIVE AMMONIA
FIXATION FROM IN NH_4Cl SOLUTIONS

| pH* | Sims silt loam | Virgin muck | Fertilized muck | Lignin |
|----------------------------|----------------|-------------|-----------------|-----------|
| | me./100g. | me./100g. | me./100g. | me./100g. |
| 4 | 3.5 | 2.2 | 8.6 | 4.1 |
| 7 | 1.0 | 3.3 | 9.4 | 4.1 |
| 9 | - | 12.1 | 14.4 | 29.7 |
| 10.5 | - | 25.4 | 27.6 | 100.4 |
| 12% NH_4OH | - | 32.4 | 35.3 | 168.0 |

*) pH was adjusted with NH_4OH and/or HCl after suspending 2 g. of material in 50 ml. of IN NH_4Cl solution.

TABLE 4.
INFLUENCE OF BASES AND CONCENTRATION OF NH_4Cl ON
AMMONIA FIXATION AT pH 7.5

| Concentration of NH_4Cl | Virgin muck | | Fertilized muck | | Lignin | |
|--|--------------------|--------------------------------|--------------------|--------------------------------|--------------------|--------------------------------|
| | NaOH * | $\text{Ca}(\text{OH})_2$ ** | NaOH * | $\text{Ca}(\text{OH})_2$ ** | NaOH * | $\text{Ca}(\text{OH})_2$ ** |
| | me./100g. | me./100g. | me./100g. | me./100g. | me./100g. | me./100g. |
| 0.05 N | 1.0 | 1.0 | 2.3 | 1.0 | 3.5 | 3.0 |
| 0.1 N | 1.5 | 1.0 | 3.0 | 1.8 | 5.0 | 3.4 |
| 1.0 N | 4.5 | 1.5 | 11.5 | 2.3 | 9.5 | 5.5 |

*) After addition of neutral NH_4Cl , pH was adjusted to 7.5 with NaOH .

**) After addition of neutral NH_4Cl , pH was adjusted to 7.5 with $\text{Ca}(\text{OH})_2$.

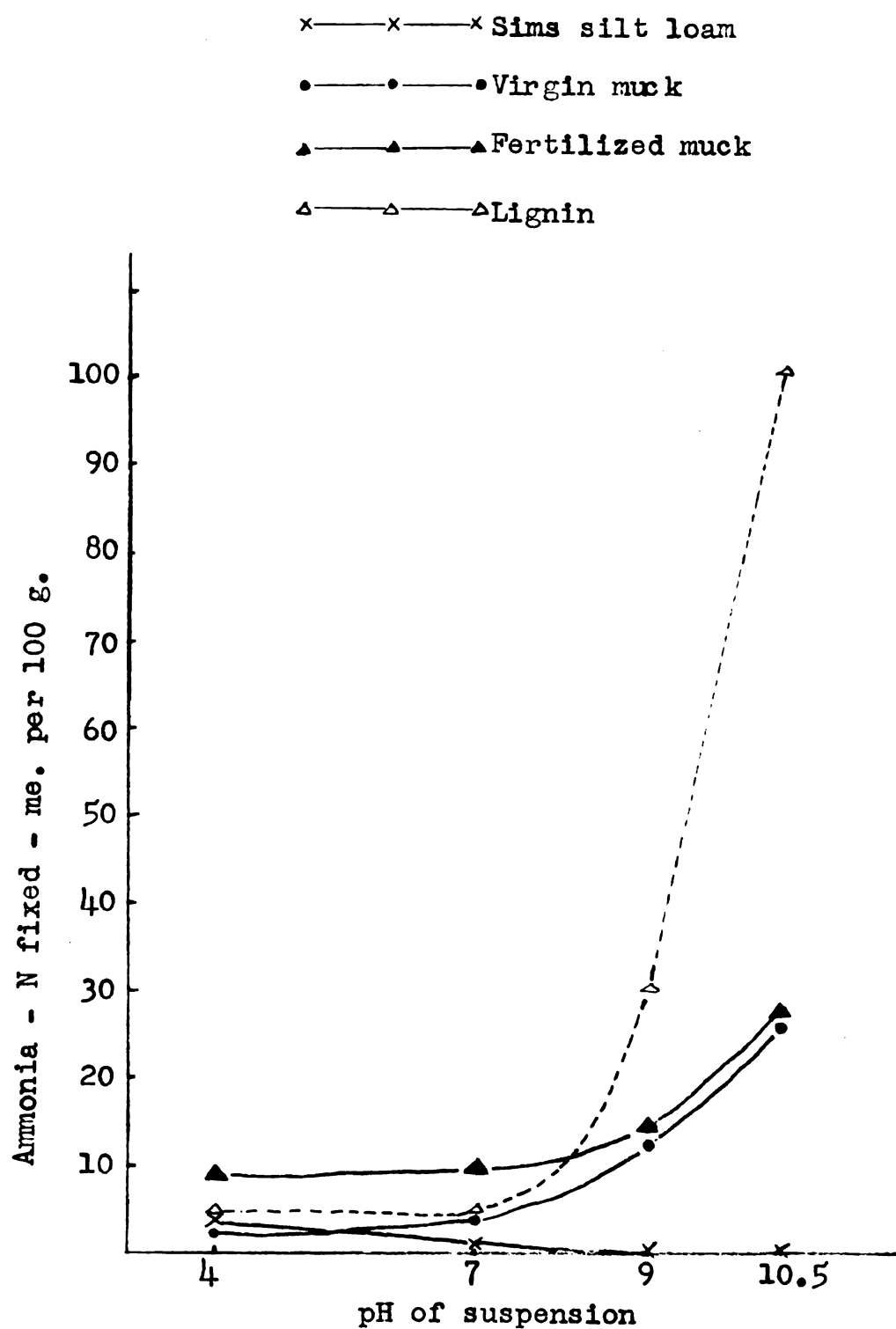


Fig. 2. Influence of pH on the oxidative fixation of ammonia nitrogen

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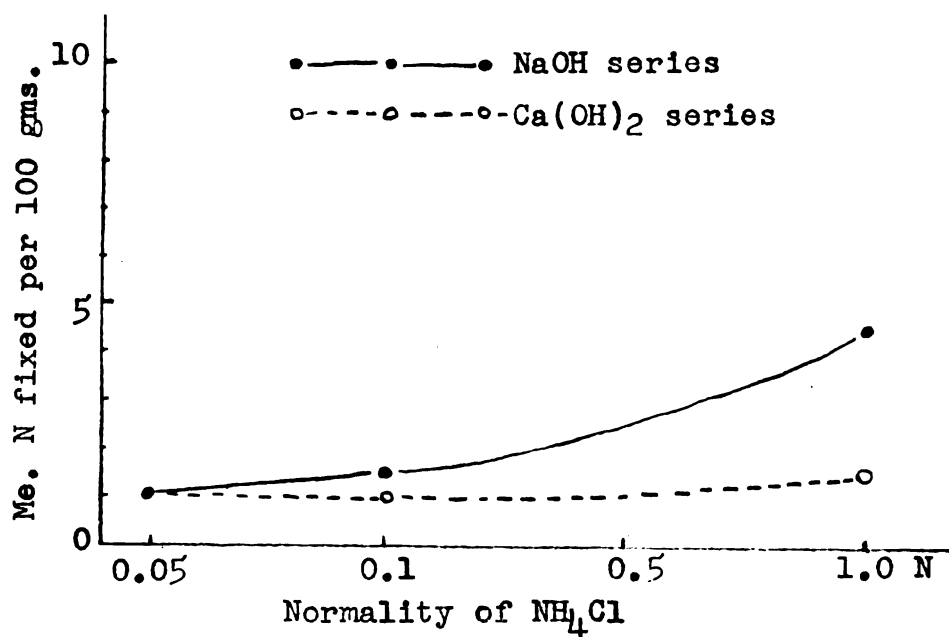


Fig. 3. Influence of bases and concentration of NH_4Cl on ammonia fixation by virgin muck at pH 7.5

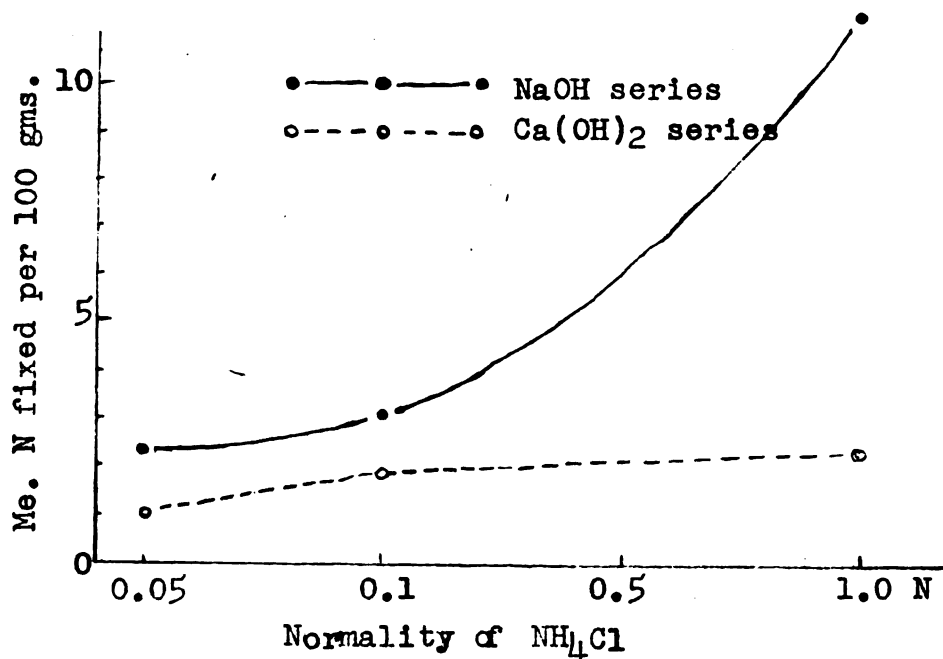


Fig. 4. Influence of bases and concentration of NH_4Cl on ammonia fixation by fertilized muck at pH 7.5

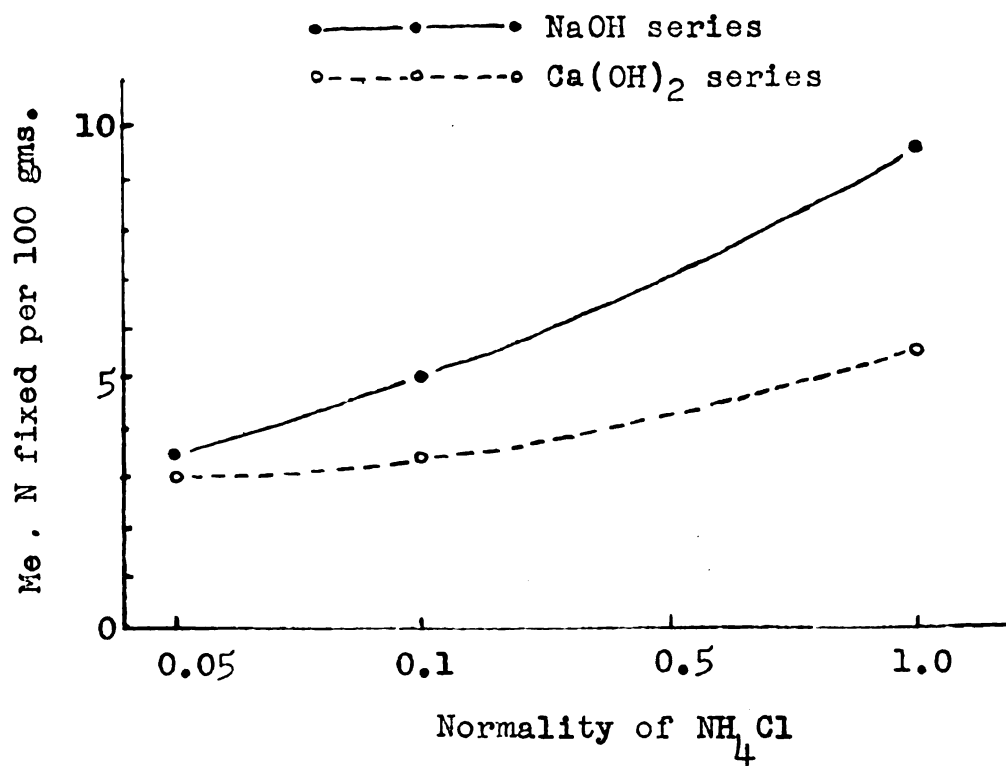


Fig. 5. Influence of bases and concentration of NH_4Cl on ammonia fixation by lignin at pH 7.5

was fixed in the presence of sodium than in the presence of calcium. This difference became greater with increasing concentration of NH_4Cl . In any case, the maximum amount of ammonia fixed was not more than 12 me. per 100g.

Influence of methylation on ammonia fixation

In another experiment, the original materials were first methylated and then subjected to oxidation in 12% NH_4OH . The results are presented in Table 5 and Figure 6. Methylation completely destroyed the capacity for oxidative fixation of ammonia by virgin and fertilized muck and resulted in a net loss of nitrogen from these materials. Although the methylated lignin fixed a small amount of ammonia, the capacity for doing so was greatly reduced, from 168.0 me. per 100g. in the original material to 9.5 me. after methylation.

Cation Exchange Capacity and Factors Affecting It

Influence of oxidation with NaOH on cation exchange capacity of the materials

Table 6 and Figure 7 show the effect of oxidation with NaOH on the cation exchange capacity of organic materials. The cation exchange capacity of all materials was increased by oxidation. In oxidized lignin, cation exchange capacity was increased from 32.9 me. in the original material to 261.1 me. per 100g. after oxidation, or about 7-fold. The increases for the two muck samples were much less. The increase for virgin muck was somewhat greater than for the fertilized reclaimed muck.

TABLE 5.

INFLUENCE OF METHYLATION ON AMMONIA FIXATION
FROM 12% NH_4OH SOLUTION

| Material | me. of NH_3 - N fixed per 100 g. | |
|-----------------|---|---------------------|
| | Original material | Methylated material |
| Virgin muck | 32.4 | -8.3 |
| Fertilized muck | 35.3 | -7.8 |
| Lignin | 168.0 | +9.5 |

TABLE 6.

INFLUENCE OF OXIDATION WITH NaOH ON CATION
EXCHANGE CAPACITY

| Material | Cation exchange capacity - me. per 100g. | | |
|-----------------|--|----------------------------------|------------|
| | Original material | After NaOH oxidation | Net change |
| Virgin muck | 142.1 | 164.1 | 22.0 |
| Fertilized muck | 161.2 | 177.6 | 16.4 |
| Lignin | 32.9 | 261.1 | 228.2 |

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- I; Amount of ammonia fixed by original sample
 II; " " " " by methylated sample
 III; Cation exchange capacity of original sample
 IV; " " " of methylated sample

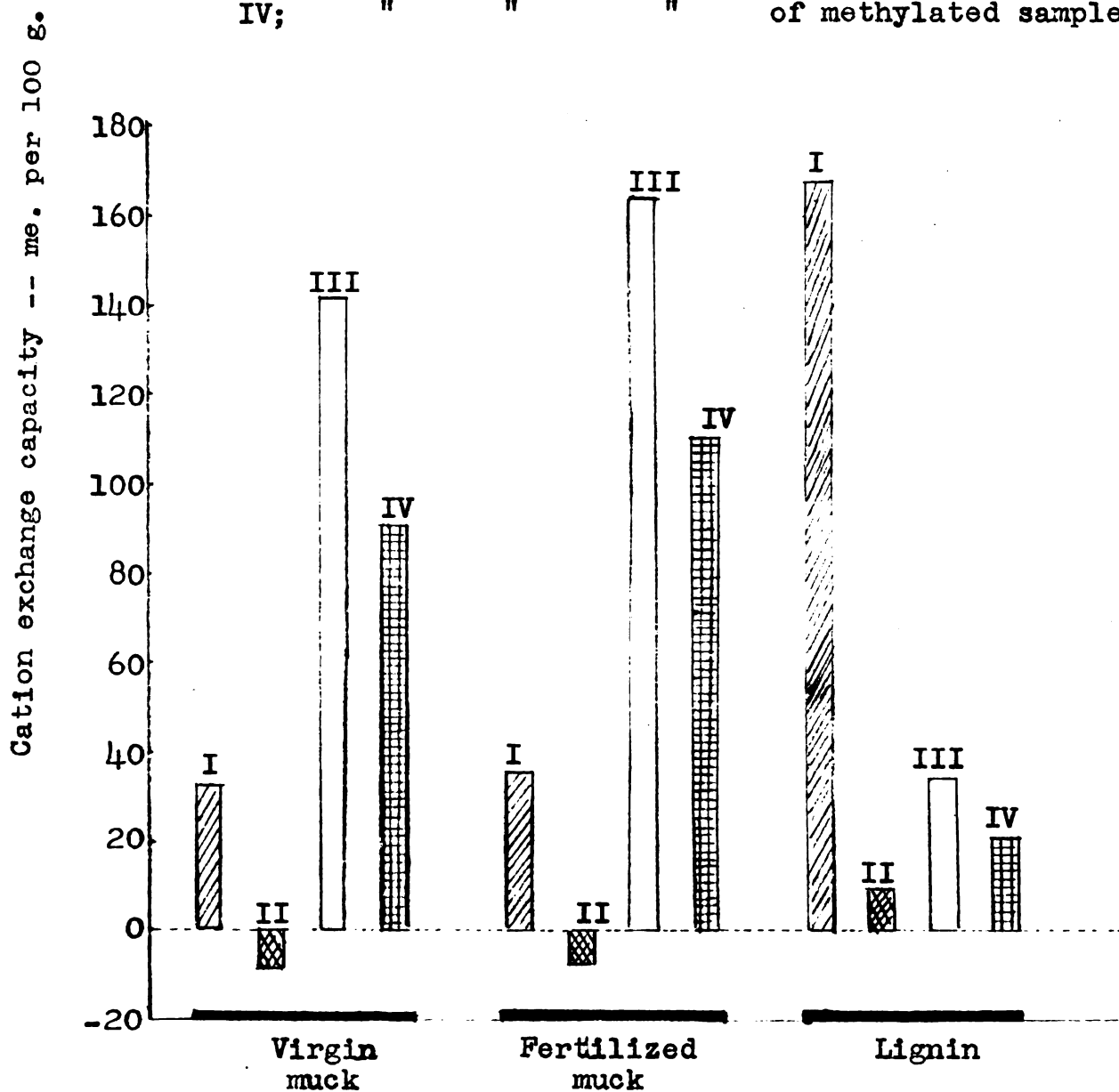


Fig. 6. Influence of methylation on ammonia fixation and cation exchange capacity

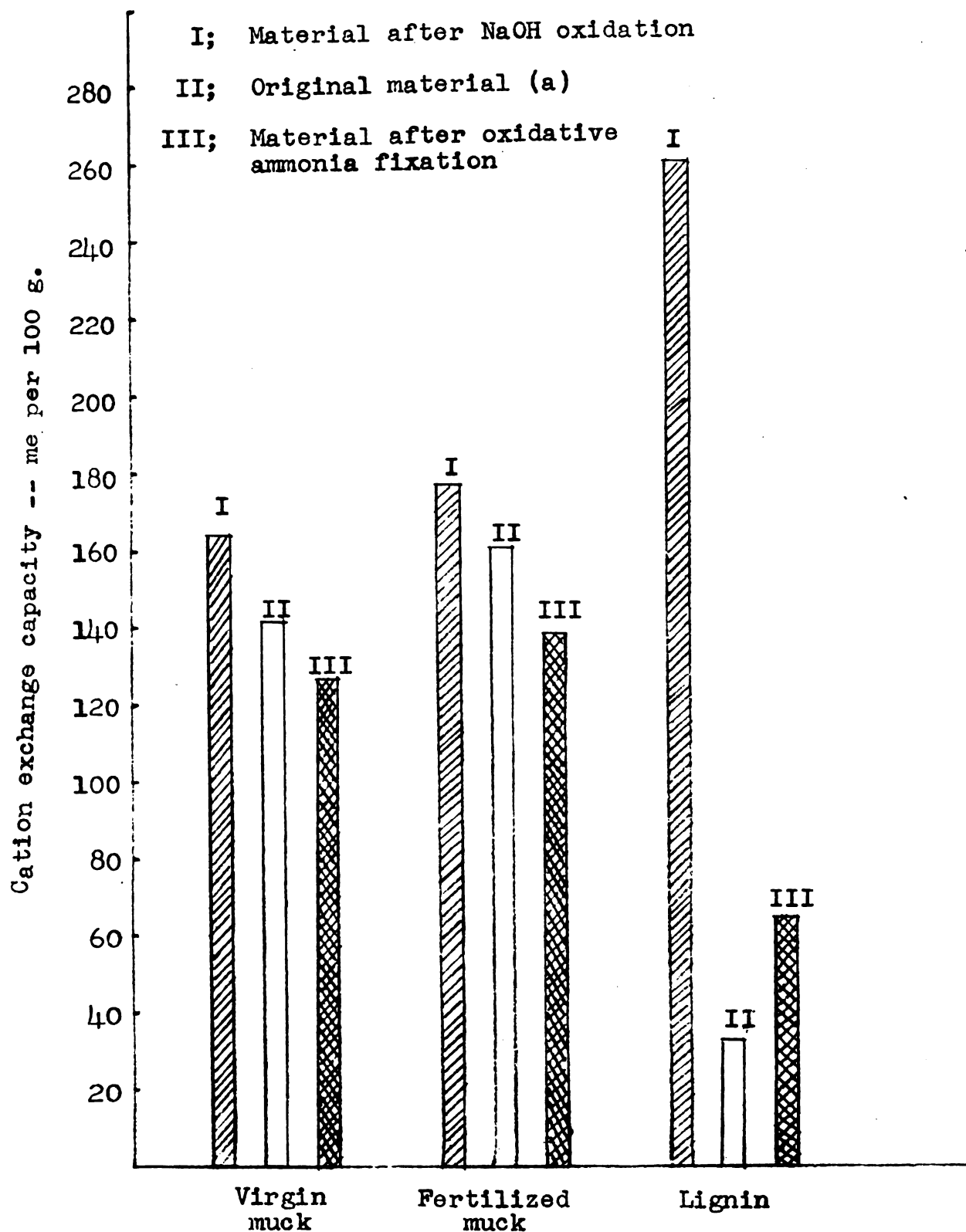


Fig. 7. Effects of NaOH oxidation and oxidative ammonia fixation on cation exchange capacity.

Influence of oxidative ammonia fixation on The cation exchange capacity of materials

The effect of oxidative fixation of ammonia from 12% NH_4OH solution on the cation exchange capacity of materials is shown by data presented in Table 7 and Figure 7. Ammonia fixation decreased cation exchange capacity of virgin muck and fertilized muck, 16.1 and 22.6 me. per 100g., respectively. On the other hand, an increase of 32 me. per 100 g. was observed in ammonia-fixed lignin. This is an approximate 2 fold increase, which is in sharp contrast with 7-fold increase in cation exchange capacity due to oxidation with NaOH .

Influence of methylation on cation exchange capacity of materials

The data in Table 8 and Figure 6 indicate that methylation decreased the cation exchange capacity of all materials by 30 to 40%.

Methoxyl Content of Material and Factors Affecting It

The methoxyl content was determined for materials after they had been subjected to various treatments, as follows: (a) original materials; (d) after oxidative ammonia fixation followed by methylation; (f) after oxidation with NaOH ; (e) after methylation; (g) after NaOH oxidation followed by methylation; and (h) after methylation followed by oxidation with NaOH . The results are presented in Tables 9 and 10.

Methoxyl content of virgin muck and fertilized muck was 1.8% and 1.4%, respectively, and was much less than that

[illegible]

100

1

TABLE 7.

INFLUENCE OF OXIDATIVE FIXATION OF AMMONIA
FROM 12% NH_4OH SOLUTION ON CATION
EXCHANGE CAPACITY

| Material | Cation exchange capacity - me. per 100 g. | | |
|-----------------|---|----------------------------------|------------|
| | Original material | After oxidative ammonia fixation | Net change |
| Virgin muck | 142.1 | 126.0 | -16.1 |
| Fertilized muck | 161.2 | 138.6 | -22.6 |
| Lignin | 32.9 | 64.9 | +32.0 |

TABLE 8.

INFLUENCE OF METHYLATION ON CATION EXCHANGE
CAPACITY OF MATERIALS

| Material | Cation exchange capacity - me. per 100g. | | |
|-----------------|--|---------------------|------------|
| | Original material | Methylated material | Net change |
| Virgin muck | 142.1 | 91.6 | -50.5 |
| Fertilized muck | 161.2 | 109.6 | -51.6 |
| Lignin | 32.9 | 21.0 | -11.9 |

TABLE 9.

METHOXYL CONTENT OF ORIGINAL AND METHYLATED
MATERIALS BEFORE AND AFTER OXIDATION

| Material | Methoxyl content - percent | | | |
|-----------------|----------------------------|------|------|------|
| | a* | f* | c* | h* |
| Virgin muck | 1.8 | 1.6 | 7.0 | 6.9 |
| Fertilized muck | 1.4 | 1.3 | 5.7 | 5.6 |
| Lignin | 12.8 | 11.6 | 23.3 | 23.1 |
| Lignin** | - | 12.1 | - | - |

- *) a original material
 f " " subjected to oxidation with NaOH
 c " " subjected to methylation
 h methylated material subjected to oxidation with NaOH

**) Lignin after three days oxidative ammonia fixation

TABLE 10.

INFLUENCE OF OXIDATION AND AMMONIA FIXATION
ON METHYLATION OF LIGNIN AND MUCK

| Material | Methoxyl content before methylation | Methoxyl content after methylation of | | |
|-----------------|--|---------------------------------------|----------------------------------|--|
| | | Original material (c) | NaOH oxidized material (g) | NH ₄ OH oxidized material (d) |
| Virgin muck | 1.8 | 7.0 | 6.8 | 6.4 |
| Fertilized muck | 1.4 | 5.7 | 5.6 | 5.3 |
| Lignin | 12.8 | 23.3 | 18.5 | 16.2 |

TABLE 11.

REMOVAL BY MgO DISTILLATION AND ACID LEACHING OF NITROGEN
 OXIDATIVELY FIXED FROM 12% NH_4OH SOLUTION

| Material | Nitrogen removed by MgO distillation | | Nitrogen removed by leaching with N HCl 10 | |
|-----------------|---|--------------|---|--------------|
| | me./100g. | % of fixed N | me./100g. | % of fixed N |
| Virgin muck | 11.1 | 34.2 | 11.8 | 36.4 |
| Fertilized muck | 12.5 | 35.7 | 12.7 | 35.9 |
| Lignin | 29.5 | 16.9 | 32.7 | 19.5 |

of lignin, 12.8%. Oxidation with NaOH did not materially affect the methoxyl content of the muck soils. The methoxyl content of lignin was reduced 1.2% by NaOH oxidation. After methylation, all materials were much higher in methoxyl content than were the original. The methoxyl content of methylated mucks was about 4 times greater and of methylated lignin about 2 times greater than in the materials before methylation. Oxidation with NaOH changed the methoxyl content of methylated materials very slightly, if at all.

Data in Table 10 show the effects of prior oxidation with NaOH or NH_4OH on the methylation of lignin and muck. When virgin and fertilized mucks were first oxidized with NaOH and then subjected to methylation, the increase in methoxyl groups was essentially equivalent to the increase when the original materials were methylated without prior oxidation. When lignin was oxidized with NaOH, its capacity for reacting with methyl groups was reduced. The reduction was even greater when lignin was oxidized in 12% NH_4OH , and a similar trend was noted for the two mucks.

Stability of Fixed Nitrogen When Subjected to Treatment with Acids or Alkali

Data in Table 11 indicate that both alkali and acid treatment of virgin muck and fertilized muck after oxidative ammonia fixation in 12% NH_4OH removed nitrogen equivalent to one third of the fixed nitrogen. However, the amount of nitrogen removed from the lignin was not more than 20%: 16.9%

by distillation and 19.5% by leaching with $\frac{N}{10}$ HCl.

Ultraviolet Absorption

In Figures 7 and 8 are shown ultraviolet absorption curves for variously treated lignin and fertilized muck. On the whole, untreated lignin produced a complicated absorbance spectrum. The original material (a) had a minimum absorbance at 262 $m\mu$. and a maximum at 283 $m\mu$. Methylated lignin (c) had a minimum at 263 $m\mu$. and a maximum at 280 $m\mu$. So, the spectrum for methylated lignin (c) was fairly similar to that for the original material (a) except that the maximum and minimum peaks were no longer so clearly defined. There was a slight shift towards longer wavelengths below the minimum absorbance point and a shift towards shorter wavelengths above that point as a result of methylation of the lignin.

Oxidation with NaOH and oxidative ammonia fixation both shifted the absorption curves towards greater wavelengths in the range below 310 $m\mu$. The curve for lignin subjected to oxidation with NaOH still had a distinct minimum at 278 $m\mu$ and a maximum at 289 $m\mu$. However, the oxidative fixation of ammonia resulted in a spectrum in which the slope reversal in the curve between these two points was completely eliminated. This difference between the curve for lignin oxidized in the presence of NH_4OH and that for lignin oxidized in the presence of NaOH is similar to the effect of methylation on the curve for lignin. The reactions between lignin and ammonia and those involved in methylation both

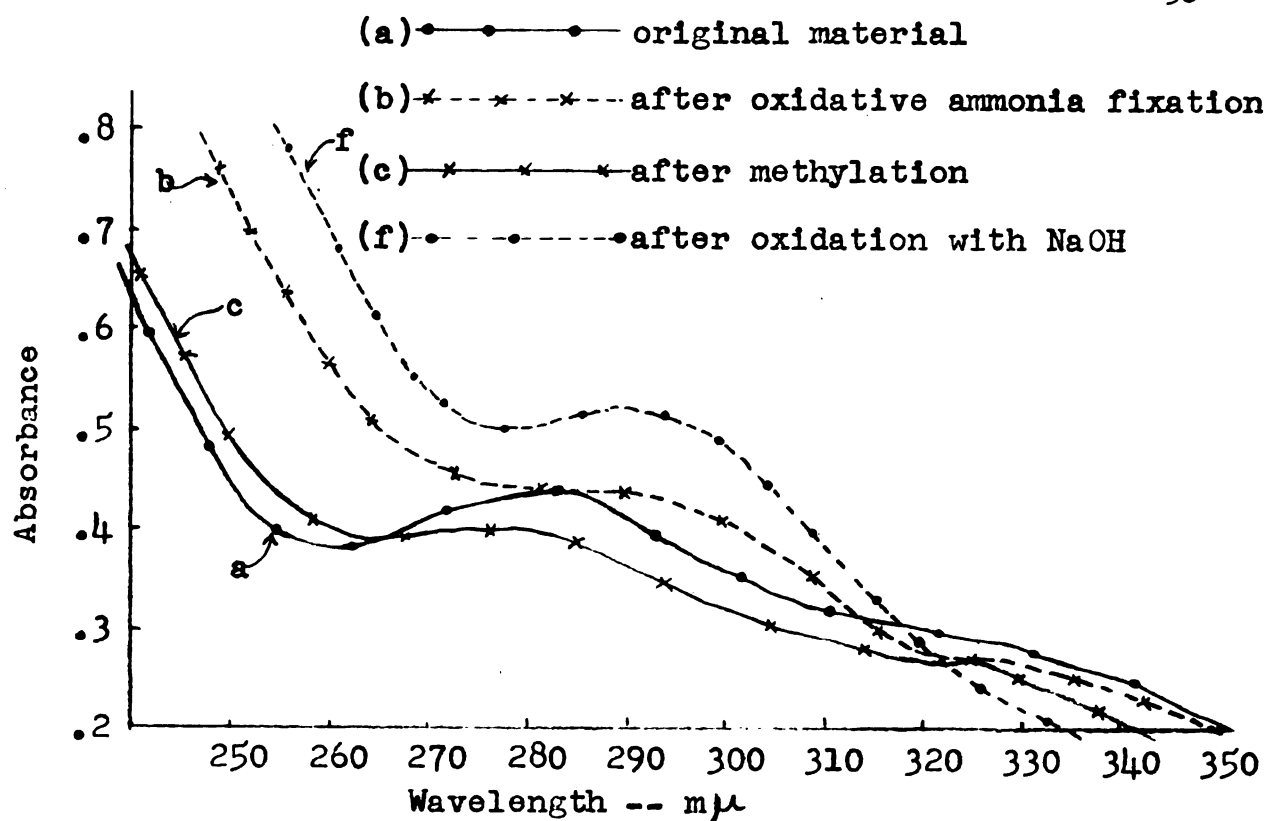


Fig. 8. Ultraviolet absorbance spectrum of lignin

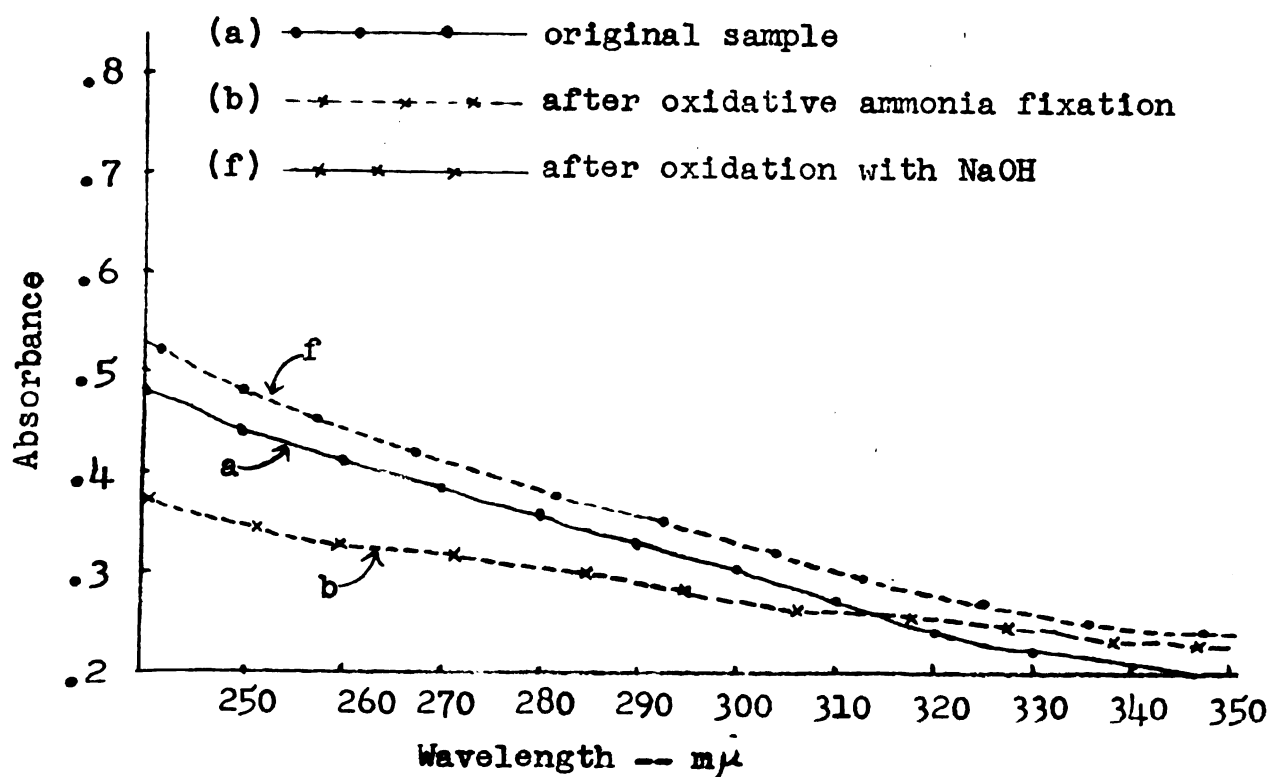


Fig. 9. Ultraviolet absorbance spectrum of fertilized muck

resulted in damping of absorbance in the range from 270 to 300 $m\mu$.

On the other hand, fertilized muck,--well-humidified matter,--produced curves with simple and gentle slopes. The effects of NaOH oxidation and oxidative ammonia fixation did not produce such striking changes in the shape of the absorbance curves as were observed in the case of lignin. Oxidation with NaOH did result in a shift toward higher wavelengths, whereas oxidation in the presence of NH_4OH resulted in a shift towards lower wavelengths. These results are similar to the relative effects of NaOH oxidation and NH_4OH oxidation on absorbance by lignin in Figure 7.

Since absorbance in the ultraviolet is principally due to resonance associated with double bonds ($C=O$, $C=C$, etc.), it would appear that oxidation per se gives rise to an increase in such double-bonded groups. On the other hand, methylation and ammonia fixation would appear to result in the disappearance of double bonds. To some extent, at least, both processes would appear to involve similar reaction sites on the lignin molecule or on humic complexes.

DISCUSSION

Direct nitration of organic materials is known to take place under drastically anhydrous conditions in the laboratory. The failure to show fixation of nitrate by lignin and soil materials under the mild conditions of the present study might have been expected. There appears to be no reason to believe that direct chemical fixation of nitrate occurs in soils under field conditions.

Numerous workers have shown that direct chemical fixation of ammonia by lignin and soil humic materials does occur and that the process is intimately associated with oxidation of the organic materials. Oxygen absorption by lignin and humic materials is promoted by alkaline conditions. Data by Junker, Bremner and others indicate that basic ions decrease in their effectiveness in promoting oxygen absorption in the order $\text{Na} > \text{K} > \text{NH}_4 > \text{Ba} > \text{Ca} > \text{Mg} > \text{Fe}$. This is also the order of their decreasing efficiency in peptization of colloidal suspensions of these organic materials, or conversely the order of their increasing effectiveness in flocculating such suspensions. Thus oxygen absorption, or the rate of autoxidation of lignin and humic materials appears to be in part a function of particle size and the active surface exposed.

In the study, much more ammonia was fixed in the presence of NaOH than in the presence of $\text{Ca}(\text{OH})_2$. This appears to have been due to the greater peptizing action of the sodium

ion and the more rapid and complete oxidation which resulted.

Ammonia fixation by the organic materials studied increased with increasing pH. The rate of increase was accelerated above pH 7.0. Oxygen absorption has also been shown to increase rapidly in the alkaline range. This may be due in part to increased peptization, but also to the activation at high pH of potential sites for oxygen absorption.

At a given pH, the fixation of ammonia was a direct function of the concentration of ammonia. The increase in fixation with increasing ammonia concentration was logarithmic rather than linear, indicating that fixation involved a series of interdependent reactions rather than a single reaction.

Methylation with dimethyl sulfate before treatment with 12% NH_4OH almost completely blocked the oxidative fixation of ammonia by lignin. A net loss of nitrogen occurred due to hydrolysis by the KOH used in the methylation procedure. Thus it appeared that ammonia fixation was specifically associated with methyl reactive groupings in these organic materials.

Methylation blocked approximately one-third of the cation exchange sites in the muck materials and in lignin. Dimethyl sulfate reacts with alcoholic-OH and phenolic-OH groups. The empirical formula submitted by the manufacturers of the lignin used, which corresponds to the formula proposed by Brauns, shows five hydroxyl groups, one of them phenolic. The actual increase in methoxyl content due to methylation

would have blocked approximately two-thirds of these hydroxyls. No carboxyl or other groups with recognized cation exchange activity are present. Thus there appears to be no stoichiometric relationship between total alcoholic and phenolic-OH groups of lignin and its cation exchange capacity.

Oxidation with NaOH resulted in a 10 to 15% increase in cation exchange capacity of the muck materials and a 7-fold increase in exchange capacity of lignin, or an increase of 228 me. per 100 g.

Oxidation in 12% NH_4OH resulted in a 10 to 15% decrease in cation exchange capacity of the muck materials. A moderate 2-fold increase for lignin was in sharp contrast to the 7-fold increase which resulted from NaOH oxidation.

Ammonium hydroxide oxidation resulted in a decrease in methylating capacity equivalent to 7.1 grams of $-\text{OCH}_3$ per 100 grams, or 229 me. If it is assumed that oxidation per se in the presence of NH_4OH resulted in loss of the same methyl reactive groupings as those affected by NaOH oxidation, then additional loss in methylating capacity with NH_4OH oxidation must have been due to the formation of non-dissociable combinations of ammonia with methyl reactive sites which were unaffected by NaOH oxidation. Significantly, the total loss of methylating capacity of lignin due to NH_4OH oxidation (229 me.) was equivalent to the total gain in cation exchange capacity with NaOH oxidation (228 me.).

By this line of reasoning, the increase in cation

100

100

exchange capacity due to NaOH oxidation can be totally accounted for as the sum of methyl reactive sites destroyed by oxidation plus ammonia reactive groups formed by oxidation at sites not active in methylation. Such ammonia reactive groups could include carboxyls resulting from oxidative degradation of saturated side chains which would not have had methylating activity. Subsequent or concurrent processes of ammonia fixation and condensation would have resulted in the tying up of an equivalent number of methyl reactive groups.

The loss of methylating capacity in lignin with NH_4OH oxidation was greater than the loss with NaOH oxidation by an amount equivalent to 2.3 grams of $-\text{OCH}_3$ per 100 grams. If the products of the reaction of ammonia with these inactivated methylation sites were all in the form of iminophenols, this would be equivalent to a loss of 74 me. of cation exchange capacity in the oxidized lignin. If linear condensation reactions occurred of the type inferred in the previous paragraph, involving side-chain carboxyls and iminophenols to form non-cyclic imides, the loss in exchange capacity would have been twice as great, or 148. me. per 100 grams. If condensation reactions involving ring closure had occurred, a third anionic grouping with cation exchange activity could have been affected for each molecule of NH_3 fixed. In this case, a total cancellation of 222meq. of exchange capacity would have resulted. The actual depression of exchange capacity due to oxidation in the presence of ammonia as

compared with oxidation in the presence of NaOH was 196 me. per 100 grams. The logarithmic increase in ammonia fixation with increasing ammonia concentration previously discussed is consistent with this concept of multiple reactions involved in ammonia fixation.

The results with lignin provide a basis for interpreting the results obtained with the muck materials. Differences in cation exchange capacity and ammonia fixing capacity of virgin muck and fertilized reclaimed muck appeared to be due to differences in degree of oxidation and degree of saturation of potential ammonia fixing sites. The fertilized reclaimed muck appeared to be more highly oxidized than the virgin muck. This was indicated by higher ash content, lower methoxyl content and lower methylating capacity. The higher cation exchange of the fertilized muck was apparently due to its higher oxidation status and the increased number of oxidized groupings (-COOH and C=C-OH) which could be inferred therefrom. Chemical oxidation in NaOH did not increase exchange capacity of the fertilized muck to as great an extent as it did in the virgin muck.

The ammonia fixing capacity was greater, particularly at low pH and with higher concentration of ammonia, in fertilized than in virgin muck. The depression in cation exchange capacity due to ammonia fixation was also greater. Thus the degree of saturation of potential ammonia fixing sites in fertilized muck was less than in the virgin muck. Apparently processes of ammonia fixation during fifteen years of

cultivation had not kept pace with processes of oxidation.

In the muck materials, all of the ammonia fixing activity appeared to be associated with oxidation of methyl reactive groups, since prior methylation completely suppressed oxidative ammonia fixation. Increases in cation exchange capacity with NaOH oxidation approached the quantitative equivalence to losses in methylating capacity due to oxidation and ammonia fixation which was found in lignin. In the virgin muck, NaOH oxidation increased exchange capacity 22 me. per 100 g. while oxidative ammonia fixation reduced methylating capacity 20 me. per 100 g. In the fertilized reclaimed muck, there was an increase of 16 me. in exchange capacity with NaOH treatment and a decrease of 13 me. in methylating capacity with NH_4OH .

Ammonia fixation, NaOH oxidation and methylation studies were not conducted on the non-fertilized muck. It had a higher ash content and a somewhat higher cation exchange capacity than the fertilized muck, which suggests that it was more highly oxidized. It had a lower nitrogen content than either the virgin or fertilized mucks. From this fact it might be expected to be lower in degree of saturation of potential ammonia fixing sites and hence higher in ammonia fixing capacity than the other two muck materials.

The data on losses of fixed ammonia with MgO distillation and acid leaching indicate that added ammonia is fixed less tenaciously by humified soil materials than by fresh

lignin. This is doubtless correlated with the higher degree of saturation of potential ammonia fixing sites in humified materials.

The differences in exchange capacity and ammonia fixation noted between muck and lignin appear to be essentially due to differences in degree of oxidation and degree of saturation of potential ammonia-fixing sites. Ultraviolet absorbance studies showed a shift towards higher wavelengths and an accentuation of characteristic minimum and maximum peaks in the curves for lignin after NaOH oxidation. Oxidative ammonia fixation of lignin tended to eliminate characteristic maxima and minima. Absorbance spectra for mucks showed gentle curves without maximum or minimum peaks. The shape of the curves for muck materials were unaffected by NaOH oxidation or ammonia fixation. Methylation had no effect on the shape of curves for lignin or for mucks.

SUMMARY

The non-biological fixation of nitrate and ammonia by mineral and organic soils and by lignin was studied in the laboratory. The results of these studies may be summarized as follows:

1. No nitrate was fixed at any pH by any of the materials. A small amount of ammonia was fixed by mineral soil at pH 4.0, very little at pH 7.0, and none above neutrality. Fixation of ammonia by organic soils and lignin increased with pH at a fixed concentration of NH_4Cl and with concentration of NH_4Cl at a fixed pH. Fixation was much greater in the presence of NaOH than when $\text{Ca}(\text{OH})_2$ was used to adjust pH.
2. Leaching with N/10 HCl and distillation with MgO removed 34 to 36% of fixed ammonia from mucks and 16 to 19% from lignin. There was little difference in the proportion removed by acid leaching or by MgO distillation.
3. Fixation of ammonia by lignin and muck soils was related to the oxidation of methyl reactive groupings to groupings active in cationic exchange, in the following manner: When parallel samples were oxidized in NaOH and in NH_4OH , an increase in cation exchange capacity was observed in NaOH oxidized samples which was equivalent to the loss in the methylating capacity of samples oxidized in NH_4OH . Each molecule of ammonia fixed, however, resulted in the inactivation of 2 to 3 of the cation exchange sites which were formed by oxidation. In the muck soils this resulted in a net loss

in cation exchange capacity as the result of oxidative fixation of ammonia.

4. Differences in cation exchange capacity and ammonia fixing capacity of virgin and cultivated mucks appeared to be related to differences in degree of oxidation and in degree of saturation of potential ammonia fixing sites. The cultivated muck was higher in ash, lower in methoxyl content, lower in methylating capacity, and higher in cation exchange and ammonia fixing capacity than the virgin soil.

5. Ultraviolet absorbance spectra for lignin exhibited complicated curves with steep slopes and a distinct minimum at 262 mu and a distinct maximum peak at 283 mu. Oxidation with NaOH resulted in a shift towards higher wavelengths of these characteristic minimum and maximum peaks; ammonia fixation tended to eliminate them. Methylation had no effect on the shape of the lignin curve. Curves for muck materials displayed gentle slopes without maximum or minimum peaks and their shape was unaffected by NaOH oxidation, ammonia fixation or methylation.

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