CHARACTERISTICS OF SOIL LEACHATES COLLECTED UNDER EASTERN HEMLOCK (<u>TSUGA</u> <u>CANADENSIS</u>)

by

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ABSTRACT

Hemlock and beech leachates which had percolated through the forest canopy and through the organic layer and the A horizon of two podzol soils were studied. Evidence was obtained to support the view that the podzolization process is more active under a conifer (hemlock) than under a deciduous (beech) canopy. It was found that the organic matter in the hemlock leachates was similar to the organic matter deposited in the B horizon of the soil studied. A chemical fractionation procedure was developed which isolated, from both hemlock leachate and B horizon extract, a substance that was potentially active in podzolization. Amino acids and phenolic materials were detected in the hydrolyzates of hemlock leachates and B horizon extracts. Phenolic acids produced by alkali fusion of hemlock leachates were identified. There was evidence that chlorogenic acid, as such, is not an important factor in podzolization of soil under hemlock canopy.

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INTRODUCTION

Since 1947, work has been conducted at Macdonald College to investigate the mechanism of podzol formation. Particular attention has been paid to the mechanism of the translocation of iron in the profile. Early workers have studied the decomposition of litter of the forest floor and the characteristics of leachates passing through this litter (17) (43) (45) (46). Work was also done on the capacities of aqueous leaf extracts and leaf leachates to react with iron and on the nature of this reaction (59) (21). Many others have studied the podzolization process by means of investigating leaf extracts (11) (41) (42) (67) and others by investigating the organic matter extracted from the podzol soil (71) (20) (53). It was decided that in order to study podzolization under more natural conditions, it would be necessary to examine organic matter present in the actual process of podzolization. It seemed that the logical place to find this organic matter would be in solutions resulting from natural rainfall that were leaching through the soil and had just reached the bottom of the A_2 horizon of the podzol. In order to collect these solutions, trays were set up containing the A horizon of the soil from the organic layer to the bottom of the A_2 horizon in such a way that natural rainfall passing through them could be collected. This thesis describes investigations on the nature of the organic matter in these solutions.

REVIEW OF LITERATURE

According to Joffe (34), in a review of the podzolization process, Sprengel described, over a hundred years ago, what we now know as podzol soils. At that time many believed that the ash-grey to white horizon found in podzols is not formed in situ, but it had been brought in by winds or laid down by water. Muller, and later Ramann, refuted the idea of the white layer being an independent geological stratum but did not explain its origin. It was not until the Dokuchaev school of soil science revealed the genetic relationship between the soil horizons that it was possible to elucidate the mode of formation of the bleached layer in the forest soils of the temperate region and the north.

Podzols are a Great Soil Group that occur widely in Russia, Northern Europe and North America. They are typically developed under coniferous forests but have been found under numerous deciduous trees and under heath vegetation. Podzols are characterized by iron movement in the profile and a bleached layer. The profile has a strong acid reaction. It was due to the bleached layer that the profile acquired its name from the Russian word "podzol" which signified an ash-like soil.

The typical podzol profile has several distinct layers or horizons. The top layer, the Ao horizon, is essentially organic and is dark brown in colour.

The next horizon, just below the Ao, is the A_1 and is the beginning of the mineral soil. It, too, is dark in colour and has a high content of organic matter. Russell (58) states that the thickness of the Ao horizon relative to the A_1 horizon varies considerably. Under heath and some forests the Ao can be thin and the A_1 thick; whereas, under well developed podzols under coniferous forest on sandy soils the A_1 is absent.

The next horizon is the A_2 which is the bleached layer. The organic content is relatively low in this layer and sesquioxides have been leached out.

Next is the B horizon which is enriched with some of the materials that have been leached from the above horizon. The constituents most frequently accumulated are organic matter, sesquioxides and clay. The colour of the B horizon may vary from a rust to a dark brown. According to Russell, the A_2 and B horizons also vary considerably, often over a small area. The boundary between them also is irregular. The depth of the profile from the top of the Ao to the bottom of the B horizon can vary from a few centimeters to over a meter.

The C horizon is next and is considered to be the parent material.

Joffe (34) has reviewed the work done up until 1948 on the process of podzolization. According to Joffe, in all the earlier studies on podzol soils, the process of podzolization has been linked with the soil organic acids and organic matter in general. Much of the work reviewed by Joffe suggests that it is organic matter in true solution as organic acids or in colloidal solutes that leaches sesquioxides from the A_2 horizon and carries them down the profile. It is this leaching of sesquioxides, particularly of oxides of iron, and the failure of dark-coloured organic matter to accumulate in it, that gives the A_2 horizon its bleached colour.

Many papers have been published since 1948 on the podzolization process. However, the mechanisms of podzolization still remain unclear. It is still generally considered that the organic matter leached from the forest litter is responsible for the leaching of the A_2 horizon.

This review of literature will present some of the later work that has been done on the processes of podzolization. Particular attention will be paid to the constituents of organic matter believed to be active in podzolization and their chemical nature.

The crux of the problem, according to Russell (58), is what fraction of the organic matter is responsible for the movement of iron and aluminum in the profile? Two processes are thought to occur. First, finely divided humus particles can mobilize the hydrated ferric oxide formed during the weathering of the soil. Secondly, polybasic carboxylic acids are the principal agents for the movement of iron and the aluminum. It is known that oxalic, malic and tartartic acids are capable, even in weak solutions, of bringing iron and aluminum into solution in the form of co-ordination compounds. Russell stated that their presence has only been assumed and not actually demonstrated in the downward moving water. He suggested that the finely dispersed humic acids may also mobilize the iron by the formation of co-ordinate complexes with their carboxylic acid groups in which case the mechanism would be the same for both processes.

Deb (20) reviewed the proposed mechanisms for movement of iron in podzol profiles and dismissed all but two of them. He thought that iron moved either as a negatively charged humic-protected iron oxide sol or as complex metallo organic ions. Deb proved that complete peptization of iron oxide could be obtained at an iron oxide/humic acid ratio of approximately 3:1 at a pH value of 4.0. He also found that a higher pH values less humic material was necessary for peptization. He concluded from these findings that there was enough humus in the soil solution to fully peptize any iron oxide sol formed by weathering in the upper horizons of a podzol and to carry it down in the percolating water.

Stobbe (63), working with leaf leachates which were collected from natural rainfall passing through decomposing maple leaves, found that they could extract iron and aluminum from grey brown podzolic soil which was alkaline in reaction and contained carbonates. These leachates removed more iron and aluminum from the above soil than from an acid soil. This is contrary to theories on podzolization which suppose that the carbonates are leached out before the sesquioxides begin to move.

Lutwick and DeLong (44) found that beech and maple leaf leachates obtained in a similar manner to that used by Stobbe, could extract iron and aluminum from both water-deposited sand and calcareous till taken from the C horizon of a podzol and a brown forest soil, respectively. In agreement with Stobbe, they found that the leachates extracted more iron from the calcareous till than from the podzol soil. The beech leachate lost aluminum to these soils whereas the maple nearly always extracted it. Both kinds of leachates extracted calcium from the brown soil and lost calcium to the podzol soil.

In similar leachates, Boswall (17) found small amounts of citric, malic and oxalic acids. McKinley (46), who continued the investigation of the organic acids in leachates from decomposing leaves initiated by Boswall, also proved the presence of phosphoric and sulphuric acids in addition to those proved by Boswall, but concluded that these were present in such small amounts that it was unlikely that they played a significant role in podzolization.

Lossaint (41) (42) reported that sterile aqueous extracts of eleven forest litters were able to dissolve ferric oxides from soils. Extracts from coniferous forest litter were more active than extracts from deciduous litter. Titratable acidity and the presence of tannins gave a good correlation with ability to dissolve the sesquioxides.

Thorp (65) showed that appreciable quantities of calcium, magnesium, iron and manganese were mobilized by passing leaf extracts (oak, beech, ash and hickory) and organic acids (oxalic and tannic) through columns of soil. The organic acids produced visibly bleached "A horizons" and "B horizons". Maple leaf extracts produced about the same blue-grey "gleyed effect" as tannic acid. Extracts from hickory, oak and beech gave weakly bleached "A horizons".

Bloomfield (5) (6) (7) (8) (9) (10) (11) (12) (13) (14) (15) has done considerable work with aqueous leaf extracts and their abilities to mobilize iron and aluminum oxides. He found that aqueous extracts of picked Scots pine needles, kauri litter, picked and fallen larch needles, fallen aspen, and fallen ash leaves were capable of bringing into solution large amounts of iron and aluminum from co-precipitated iron and aluminum oxides. This mobilization took place over a wide pH range (4-7) and under anaerobic and aerobic conditions. Both lowering the pH and having aerobic conditions decreased the amount of iron in solution. The effect of pH and aeration varied considerably with the kind of extract. The results were the same for the solution of aluminum but changing the pH and the aeration made smaller differences in the amount of aluminum mobilized.

Bloomfield claimed, since he found large amounts of ferrous iron in the extracts, that the solubilization of ferric oxide by the extracts involved the reduction of the iron to the ferrous state. He believed that the reducing compounds were polyphenols.

From the results of these experiments, Bloomfield proposed a mechanism for the formation of the A_2 horizon of podzols. He thought that percolating rainwater leached materials from the fallen leaves and that this solution dissolved, reduced and complexed the sesquioxides and thus moved them out of the A_2 horizon. Bloomfield said that in the earlier work in podzols, the process of podzolization was associated with the type of podzols found; that is, they were strongly acid in nature and had considerable raw humus. The fact that the podzolization process can take place in slightly acid and in some cases alkaline soils shows that an acid environment is not essential. This is in agreement with his work with leaf extracts which were able to dissolve ferric and aluminum oxides at a neutral pH. Stobbe (63), Lutwick and DeLong (44) also found that leaf leachates could extract iron and aluminum from a calcareous till. Bloomfield stated that it was possible that the more transitory constituents of the plant debris and its relatively simple decomposition products, rather than humic compounds, were responsible for the podzolization process.

DeLong and Schnitzer (21) (59) have also done extensive work with leaf extracts of finely ground air dried leaves of the autumnal leaf fall and leachates from decomposing leaves. They were mainly interested in the capacities of these solutions to react with iron. The extracts were obtained by making water extracts of various fall gathered air dried leaves. Their leachates were obtained from natural rainfall leaching (a) tree-tops in leaf, (b) leaves of the autumnal leaf fall and (c) the combined Aoo, Ao, and A_2 horizons of a podzol profile beneath beech and beneath pine-hemlock canopy.

They measured the capacities of these solutions to hold iron in solution when added as ferric nitrate, at a constant pH value. They found that all these solutions were capable of holding large amounts of iron in suspension and that this capacity increased with increasing pH. The amount of iron held by an extract of poplar leaves at a pH of 8.5 using NaOH as the base approached the proportion of 1:1 of iron to organic matter. It was concluded that this high value could only be explained by the organic matter acting as a protective colloid.

The capacity of these solutions to take up iron from freshly precipitated ferric hydroxide was also measured. All of the solutions took up iron, but it was much less than the amount of ferric iron that the solution could contain in suspension. Raising the pH of a poplar extract from 5.5 to 7.5 doubled the amount of iron taken up. Shaking the solution also increased considerably the quantity of iron in solution. In contrast to Bloomfield, they found that most of the iron was in the ferric form.

Generally the leachates were much more active than leaf extracts in holding iron in solution and in mobilizing iron from ferric hydroxide.

It was possible to isolate a fraction of organic matter from aqueous extracts of poplar leaves and from leachates obtained in the field from poplar leaves, in the first and second year of exposure, by adding ethanol to these solutions to a concentration of 80 per cent. This procedure precipitated out a fraction which they called the AIF (alcohol insoluble fraction). The AIF fractions had characteristics which would indicate that the major component in them was an acidic polysaccharide. These fractions possessed the major part of the capacities of these solutions (50 to 85 per cent) to react with iron while only containing 20 to 50 per cent of the organic matter. Potentiometric titrations of the AIF fractions in presence and absence of ferric iron failed to reveal evidence of chelation. One of the conclusions drawn from this work by the authors was that both the forest canopy and the forest floor contributed solutions capable of the mobilization and transportation of iron under favourable conditions.

Much of the literature reviewed so far has been concerned with the ability of aqueous leaf extracts and leaf leachates to mobilize iron from soils and ferric oxide. It has been clearly shown that these solutions can mobilize iron in large amounts. However, little information is available on the chemical nature of the organic matter active in podzolization. The leachates studied at Macdonald College should resemble fairly closely the materials that are passing from the litter of a podzol. Boswall (17) and McKinley (46) have demonstrated that citric, malic and oxalic acids exist in leachates in small amounts. DeLong and Schnitzer (21) have found in leachates a fraction resembling an acidic polysaccharide. Much work has been done with organic matter extracted from soils. Whether organic matter extracted from soils resembles that passing through the A horizon is open to question. However, a brief review of recent concepts of the nature of the organic matter of soils may be appropriate.

By the classical method of extraction of organic matter from soil by alkali, two fractions are obtained: (a) humic acid which is soluble in alkali and insoluble in acid, (b) fulvic acid which is soluble in alkali and soluble in acid. Work on both these fractions will be discussed.

Humic acid or humus has been reviewed by Waksman (69). At the time of this review the majority of investigators believed that humic acid was derived from lignin as a complex of lignin and protein.

Bremner (18) has reviewed the recent literature on soil organic matter up to 1953. He has concluded that there are two distinct theories regarding the origin of the humic substances of soil: (a) the humus is derived from the alteration of plant lignins entering the soil, (b) the humus is a product synthesized by, or formed by, autolysis of soil microorganisms.

Gottlieb and Hendricks (28) used the newer techniques of lignin chemistry to study the humic fraction of the soil. The alkaline nitrobenzene oxidation of a muck soil and of an alkaline extract of a soil yielded various amounts of aldehydic, acidic, and neutral oils. No definitely characterizable products could be obtained from these oils. The application of hydrogen under high pressure and high temperature to various organic matter preparations resulted in hydrogenation and hydrolysis of a large part of the organic matter into colourless oils. These were probably mixtures of saturated aromatic alcohols. Gottlieb and Hendricks compared the results of hydrogenation of materials derived from soils with those obtained from the hydrogenation of "alkali lignin" and from the general similarities of the products concluded that lignin in the soil goes through a similar type of change, at a lower pH and a slower rate, than does lignin that has been treated with alkali. This change consists of a condensation of the lignin molecule with the production of fused rings. These fused rings are more resistant to hydrogenolysis than unaltered lignin. They concluded that the material derived from plant lignin in the soil is drastically altered in the kind and position of the peripheral groupings on the aromatic rings.

Sowden and Atkinson (60) treated a soil humic fraction by a method used to obtain lignin from plant material. The products obtained were different in many respects from lignin. The soil fraction was much lower in methoxyl content than the fraction obtained from plant material and higher in nitrogen content. It was soluble in water and insoluble in acid solutions, even when the acid concentration was weak. They found, however, that the ultra violet absorption curve of the material studied was similar in many respects to that of the "lignin" fraction obtained from plant material.

This could indicate the aromatic character of the isolated material.

Morrison (48) reported from the results of oxidizing soil organic matter with alkaline nitrobenzene that there were significant amounts of syringyl, guaiacyl and p-hydroxyphenyl residues which were almost certainly derived from lignin. The yields of the aldehydes of the above materials from mineral soils accounted for about 0.5 to 1.0 per cent of the total organic carbon and from peats for about 1 to 4 per cent.

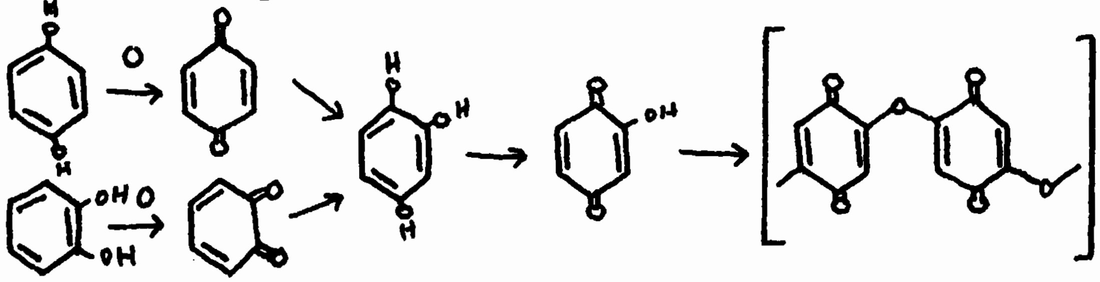
Many investigators are of the opinion that the humus of soils is not derived from lignin. Aleksandrova (1) reported that the humus fraction need not necessarily be associated with lignin due to the fact that algae, lichens and mosses which do not contain lignin give rise to humus.

Laatsch (38) concluded from his experiments that many organisms, especially fungi and actinomycetes, produce quinone derivatives "in vivo", and that these react with amino acids to give humus.

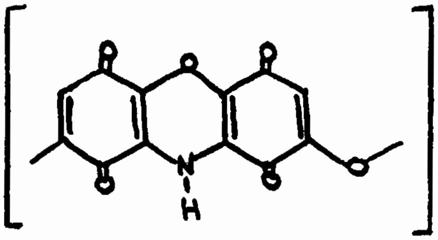
Kononova (36) (37) claimed that the view developed by Waksman (69), that humus is a ligno-protein, has been abandoned and has been replaced by the theory that humus consists of a group of high molecular condensation products such as humic, crenic, and apocrenic acids. This view has been mainly developed by German and Russian workers. Kononova believed that the formation of humic acid is a process in which all constituents of plant residues and their decomposition products interact with products of microbial metabolism. The results from her data suggested that fundamentally the humic and fulvic acids are similar except for their degree of polymerization. The humus of chernozems have the most highly condensed humic acids whereas the podzols have the least. She believed that the fundamental structural unit is a flat net of polymerized cyclic carbon with side chains of linear polymerized carbon. The low humic acid content of the podzols is due to the large hydrolytic effects; whereas with chernozems, the condensation process proceeds with less hydration. Kononova and Aleksandrova (35) have recently found that *Penicillium* and *Asperillus niger*, grown on inorganic materials and glucose, produced a cherry red solution which had some of the properties of soil organic matter. They detected aromatic compounds in the solution. Enzymes of the phenoloxidase types were found. They believed that these catalyzed the condensation of aromatic compounds with organic nitrogen. The kinds and amounts of amino acids obtained from newly formed material and from humus from soil were similar.

Pochon and Wang (52) found that during the metabolism of benzoic acid by *Azotobacter* a substance was formed that resembled soil humus. They believed that the oxidation of benzenoids by *Azotobacter* is one cause of the formation of humus in the soil.

Several investigators have produced humic-like substances by chemical methods. Cornec and Brusset (19) have found that hydroquinone on oxidation with sodium persulphate gave a product that resembled humic acid. Flaig (24) has found that solutions of para and ortho dihydroxy benzenes were oxidized by air at a pH of 8 to 9 and polymerized to model humic acids according to this scheme:



Resorcinol did not react under the same conditions. In the presence of NH_4OH at a pH of eleven, nitrogen was introduced. This gave a polymer containing 5.65 per cent nitrogen as shown on the following page.



The nitrogen was resistant to distillation with concentrated NaOH. Flaig and Beutelspacher (23) found no difference in appearance between the natural and synthetic-phenolic humic acids when viewed with an electron microscope. No evidence of crystalline structure could be found with either the synthetic or natural humic acids. Flaig and Schulze (22) found that the synthetic humic acids prepared from hydroquinone were the closest to the natural humic acids. Flaig and Schulze consider it proved that the formation of humic acid goes through an oxyquinone state.

Much less work has been done on the fulvic acid fraction of soil matter than with the humic acids. Tiurin (66) (67) found that water soluble humus from the Ao and A_1 horizons of a strongly podzolized soil had equivalent weights of 235 and 200, respectively. The high content of carbon and the high degree of oxidation resembled fulvic acid. He concluded from this that the principal part in podzolization is played by organic substances having the properties of fulvic acids. Ponomareva (53) extracted fulvic acid from the B horizon as an ammonium salt. The acid had an equivalent weight of 160. The solution was separated into two fractions by the addition of barium or calcium hydroxide. The soluble fraction was lighter in colour and contained more carbon. Forsyth (25) has developed a method for the fractionation of fulvic acid. This was achieved by a selective absorption technique on charcoal. The solution of fulvic acid was passed through a charcoal pad and washed with 0.1 N HCl. Fraction A was comprised of the filtrate and washings. Three more fractions were obtained by eluting successively with an aqueous acetone solution containing 90 per cent acetone, distilled water and 0.5 N NaOH. These fractions were called B, C and D, respectively. Fraction A was thought to have contained many simple organic substances (amino acids, purine bases, sugars, etc.). Seventy to eighty per cent of the total nitrogen was found in this fraction. A powder isolated from fraction B had many properties of a phenolic glycoside. By adding three volumes of acetone to fraction C, a white precipitate occurred which was shown to be comprised of polysaccharides containing uronic groups. Fraction D was rich in nitrogen and phosphorus and gave a positive test for pentoses.

EXPERIMENTAL MATERIALS AND METHODS

Leachates were obtained from natural rainfall that had percolated through the forest canopy and through the organic layer and the A_2 horizon of two podzols. The A horizons were obtained by cutting out from beneath the appropriate canopy four sections each measuring approximately 1 foot by 3 feet by 6 to 9 inches to fill a tray. These sections were fitted into each tray tightly enough so that the rainfall would pass through rather than around them. The trays were specially designed to collect the natural rainfall percolating through the A horizon of the soil. Each tray was 4 feet by 3 feet by 10 inches with a bottom sloping towards the centre and the front terminating in a pipe connected to a covered pail. The trays were set into the ground with their tops slightly above ground level under the appropriate canopy, and covered with 1/4 inch mesh wire screens. The surfaces of the trays, screens and pails were coated with asphalt paint.

Canopy drip solutions from hemlock trees were collected in similar trays to those holding the A horizon of soil by placing them under a hemlock canopy. Each year 0.5 pounds of beech leaves were added to each tray containing the A horizon of the soil obtained under beech canopy. This operation was not necessary for the hemlock A horizons since the natural fall of hemlock needles could penetrate the mesh screens.

The A horizons were obtained from two locations on St. Amable loamy sand occupied by beech (Fagus grandifolia) and hemlock (Tsuga canadensis) stands respectively. This soil has been described by Lajoie and Baril (39). The soil under the beech is a moderately well drained podzol while that under the hemlock is less well drained. However, there is little difference in the drainage of the two profiles above the lower part of the B horizon. The profile under hemlock has a thicker and more consistently developed A_2 horizon. Four trays of each type of A horizon were set up adjacent to the respective areas from which the A horizons were removed. The A horizon samples were placed in the trays on May 12th and 13th, 1955 and have remained there since that date. Leachates obtained under beech canopy from A horizons taken from beneath beech canopy are called beech leachates and those obtained under hemlock canopy from A horizons taken under hemlock canopy are termed hemlock leachates.

Hemlock needle extracts were prepared by shaking 4 gm. of ground hemlock needles in 100 ml. of water for thirty minutes. The hemlock needles were obtained from hemlock branches that had been cut from live trees. When the twigs were stored in a dry place, the needles fell from them in a few days without any visible change in colour. The needles were air dried and ground.

Organic matter was extracted from a sample of the B_{g1} horizon of the soil under hemlock canopy obtained near the site where the A horizon was removed. This was achieved by adding 20 gm. of the soil, which had been ground to pass through a 20 mesh sieve, and 20 ml. of Dowex-50 cation exchange resin (H form) to 150 ml. of water. A small amount of chloroform was added to prevent any microbial activity. These suspensions were then shaken for various times up to ninety-six hours. The resulting aqueous solution was then separated from the soil and resin by filtration. It was possible by this method to extract, at the longest period of shaking, up to 48 per cent of the organic matter. The extracts mentioned later were obtained by shaking soil, resin and water in the proportions given for fifty-six hours. These extracts contained approximately 31 per cent of the total organic matter of the B_{g1} soil sample.

Preliminary Examination of Leachates

Leachates were collected after each rainfall from June 11, 1955 to August 14, 1956 that was of sufficient intensity to cause penetration through the material on the trays. The volume of leachate from each tray was recorded. The leachates were then filtered and the following determinations were carried out:

1. pH, using a Beckman Model G potentiometer with glass-calomel electrodes.

2. Total solids, determined by evaporating a portion of the leachate over a steam bath and weighing the residue after completion of desiccation at 105°C.

3. Organic matter, determined by the dichromate method (51).

4. Iron determination, made by the thiocyanate method of Houlihan and Farina (33) after a wet digestion with nitric and perchloric

acids.

Separation of Organic Matter from Leachates

Separation of most of the organic matter contained in the leachates was achieved by alternating additions of small amounts of solutions of ferric nitrate and sodium hydroxide, these additions being adjusted so as to maintain the pH near that of the leachates collected, namely about pH 4.5. The additions were continued until a precipitate formed and the supernatant was colourless. The precipitate was allowed to settle and the supernatant liquid was decanted. If the material was not needed immediately, a small amount of chloroform was added to the residual suspension and it was stored at a temperature of about 6°C.

Prior to use, the iron was removed from the precipitated material. For this purpose 100 ml. of the residual suspension were adjusted to pH 6.5 by addition of NaOH solution; 100 ml. of a one per cent solution of 8-quinolinol in chloroform were added, and the mixture was shaken on a rotary shaker. Frequent adjustments of the pH of the aqueous phase were required during the early part of the extraction of iron. After twenty-four hours of shaking, the phases were separated and the aqueous layer was extracted twice more in the same manner. Following these extractions the aqueous phase was shaken repeatedly for twelve hour periods with chloroform only until the chloroform phase no longer gave a positive test for 8-quinolinol, the ferric chloride-ferricyanide test for phenols (3) being used for this purpose. At this stage the organic matter retained in the aqueous phase represented about 90 per cent of that present in the original leachates. The concentration of organic matter in the aqueous phase was approximately twenty times the concentration of organic matter in the original leachate.

Preparation of a Hydrogen Resin

The hydrogen resin was prepared by first leaching a column of Dowex-50 cation exchange resin (Na form) with 3 N HCl. The column was then eluted with distilled water until the pH of the effluent was approximately equal to that of the original distilled water.

Iron Saturation Capacity

The iron saturation capacities were determined by the method outlined by DeLong and Schnitzer (21). The iron saturation capacity at any given pH was determined by alternating additions of small amounts of solutions of ferric nitrate and sodium hydroxide, these additions being adjusted so as to maintain the pH at the desired level. The additions were continued until a precipitate formed.

Electrophoretic Studies

Electrophoresis was carried out in a Spinco paper electrophoresis cell, Model R - Series C, Durrum type developed by Williams as described by Block et al (4). The samples were pipetted on Whatman No. 3 filter paper which had been saturated with the buffer solution. Several buffers were used: Borate at pH levels 7.2, 7.6 and 8.6; veronal buffer at pH 8.6; phosphate buffer at pH 7.6; acetate buffer at pH 3.6 and 4.4 and citrate-phosphate buffer at pH 3.3. The acetate buffer at pH 4.4 was found to be the most satisfactory. This is approximately the pH of the natural leachate. A current of 5 milliamperes was maintained which gave a potential of approximately 70 volts. The duration of the run was twenty hours. After the papers were dried, they were examined under ultra violet light. The papers were then stained with two stains: a) methylene blue (20), b) diazotized p-nitro aniline plus sodium acetate (4).

Identification of Sugars

The solutions were hydrolyzed by boiling under reflux for six hours with normal sulphuric acid. The resulting solutions were neutralized with barium carbonate using indicator paper. The barium sulphate was separated by centrifugation and the supernatant was concentrated in an evacuated vacuum desiccator over concentrated sulphuric acid at room temperature. The sugars were separated by one dimensional downward flow of benzene-pyridine-n-butanol-water (1:3:5:3 v/v) along with a mixture of known sugars on the same chromatogram. The sugars were detected by staining with aniline hydrogen phthalate (50).

Amino Acid Determinations

The solutions were hydrolyzed with 6 N HCl for twelve hours under reflux. The solutions were then evaporated to dryness in an evacuated vacuum desiccator over sodium hydroxide, taken up in water and evaporated down again. This was repeated twice more to eliminate the last traces of hydrochloric acid. The material was then taken up in a 10 per cent solution of isopropanol. The amino acids were separated by two dimensional paper chromatography. The chromatogram was run on Whatman No. 1 paper with phenol followed by n-butanol - acetic acid-water (4:1:5 v/v) organic phase (4). The amino acids were revealed by spraying with 0.25 per cent w/v ninhydrin in acetone and heating in an oven at 105°C for a few minutes (4). The amino acids were tentatively identified by comparing the pattern and colour of the spots on the chromatogram with a chromatogram that had been run with known amino acids.

Acid Breakdown Products Determinations

The hydrolyzed materials used for amino acid determinations were used here also. Chromatograms were run using the descending technique.

Small amounts of the solution were spotted on Whatman No. 1 chromagraphic papers and the papers were irrigated with n-butanol - acetic acid-water (4:1:5 v/v) organic phase, for twelve hours. After the papers were dried, they were examined under ultra violet light. For detection of phenolic materials, two stains were used: a) ferric chloride/ferricyanide (3), b) diazotized p-nitro aniline plus sodium acetate (4).

Potassium Hydroxide Fusion Studies

Fifty mgm. of organic matter, which had been taken to dryness on a steam bath, were fused in air with 3 gm. of potassium hydroxide in a nickel crucible. The cooled fused mass was then taken up in water, acidified with hydrochloric acid and extracted with ether. Purified ether was used (68). The ether solution was extracted with an aqueous solution of sodium bicarbonate. This was done to separate phenols from phenolic acids, the phenolic acids being extracted into the sodium bicarbonate solution. The acids were recovered by acidifying the sodium bicarbonate solution and extracting it with ether. The ether solution was concentrated by evaporation on a steam bath. The resulting solutions were studied by means of paper partition chromatography using the descending technique. The ether solutions were spotted on Whatman No. 1 chromatographic paper and the papers were irrigated with the following solvents: isopropyl alcohol-aqueous ammonia-water (8:1:1 v/v), benzene-propionic acid-water (2:2:1 v/v), organic phase; 20 per cent aqueous potassium hydroxide (w/v); n-butanol-pyridine-dioxane-water (70:20:5:5 v/v) (2); n-butanol-acetic acid-water (4:1:2.2 v/v) (30); n-butanol-pyridine-water (6:4:3 v/v) (54) and 2 per cent aqueous acetic acid (v/v) (30). After the papers were dry, they were examined under ultra violet light. Phenolic substances were detected by coupling them with diazotized sulfanilic acid (4), diazotized p-nitro aniline plus sodium acetate (4), diazotized p-nitro aniline plus sodium carbonate (4) and dipping in ferric chloride/ferricyanide solution (3).

Phenolic acids were isolated using a cellulose column. The ether solution was evaporated, the residue taken up in a few ml. of isopropyl alcohol-aqueous ammonia-water (8:1:1 v/v) and this solution was chromatographed on a cellulose column $\frac{1}{2}$ x 20" with isopropyl alcohol-aqueous ammonia-water (8:1:1 v/v) as a developing solvent. Ten ml. fractions were collected. A small portion of each fraction was spotted on cellulose paper and stained with a phenolic stain to locate the phenolic acids. The fractions were evaporated under vacuum to dryness. The remaining material was taken up in a solution of potassium hydroxide, acidified with hydrochloric acid and extracted with ether. The ether solution was evaporated to dryness. This procedure was repeated at least four times. Melting points and mixed melting points with authentic material were determined on the isolated material using a Fisher-Johns apparatus.

Quantitative determinations were carried out on the four phenolic acids detected on the chromatograms. The method used was essentially that of Pridham (54). The solution containing the phenolic materials was spotted on No. 1 Whatman chromatographic paper and the chromatogram was irrigated with isopropyl alcohol-aqueous ammonia-water (8:1:1 v/v). The chromatogram was dried and dipped in a solution of diazotized p-nitro aniline buffered with sodium acetate. The excess moisture was allowed to dry off and the spots were cut out in equal sizes. The coloured components were eluted with aqueous ethanolic potassium hydroxide solution in test tubes. The paper remained in the solution for twelve hours. The tubes were shaken by hand occasionally. The absorbance of the resulting solution was measured in an Evelyn colorimeter. Standard curves were prepared by chromatographing known amounts of standard compounds and carrying out the above procedure. Absorption spectra of the standard solution were determined using a Beckman Model D.U. spectrophotometer. The wave length of the maximum absorption peak was used as the wave length for the quantitative determination with the Evelyn colorimeter. It was found that after the subtraction of blanks the intensity of colour of the solution bore a linear relationship with the weight of the phenolic acids.

Hemlock Needle Extract Studies

The hemlock needle extracts were chromatographed by spotting the solution on Whatman No. 1 chromatographic paper and irrigating the chromatograms with the following solvents: n-butanol-acetic acid-water (4:1:2.2 v/v), two per cent acetic acid, forty per cent isopropyl alcohol, and water saturated ethyl acetate (4). After the chromatograms were dried, they were examined under ultra violet light, held over ammonia as a test for anthoxanthins (32), tested for catechins and leuco-anthocyanins with the vanillin test (32), tested for chlorogenic acid using the Hoepfner test (56) and stained with ferric chloride/ferricyanide (3), ferric chloride (4) and diazotized p-nitro aniline buffered with sodium acetate which are general tests for phenols.

Determination of Equivalent Weights

In the fractionation procedure, all the fractions were in the barium form at one time or another. Fraction 2 was in the barium form after precipitation from alcohol and fractions 1a and 1b (combined at this stage) were in the barium form in the initial stages of the separation. After removal of the barium with a hydrogen resin, the fractions were in the hydrogen form. Equivalent weights were determined by neutralizing the fractions to a pH of 7.0 with sodium hydroxide.

Other Methods

Aluminum was determined by using aluminon (57). Nitrogen was estimated by a micro-kjeldahl procedure (49). Carbohydrate was estimated by an anthrone method using glucose as a standard (40). Phosphorus was determined by the method of Martin and Doty (47).

EXPERIMENTAL RESULTS AND DISCUSSION

Preliminary Examination of Leachates

The values found for the concentration of organic matter in 1955 and 1956 leachates are presented in Figures 1 and 2. Inspection of the data shows that the organic concentration of the hemlock generally increases throughout the 1955 season. The increase is continued the following season making the average 1956 values higher than those of 1955.

The organic matter concentration of the beech leachate was relatively constant throughout 1955 with a possible mid-season maximum. The concentration of the leachate at the beginning of the 1956 season was relatively high, but became considerably less with the advance of the season. The concentration of the organic matter of the beech leachate was less than the hemlock throughout both seasons.

It must be emphasized that the data given in Figures 1 and 2 are mean values for the leachates obtained from four trays of beech A horizon and four trays of hemlock A horizon, and that the difference between the mean values is relatively small. The organic matter concentrations

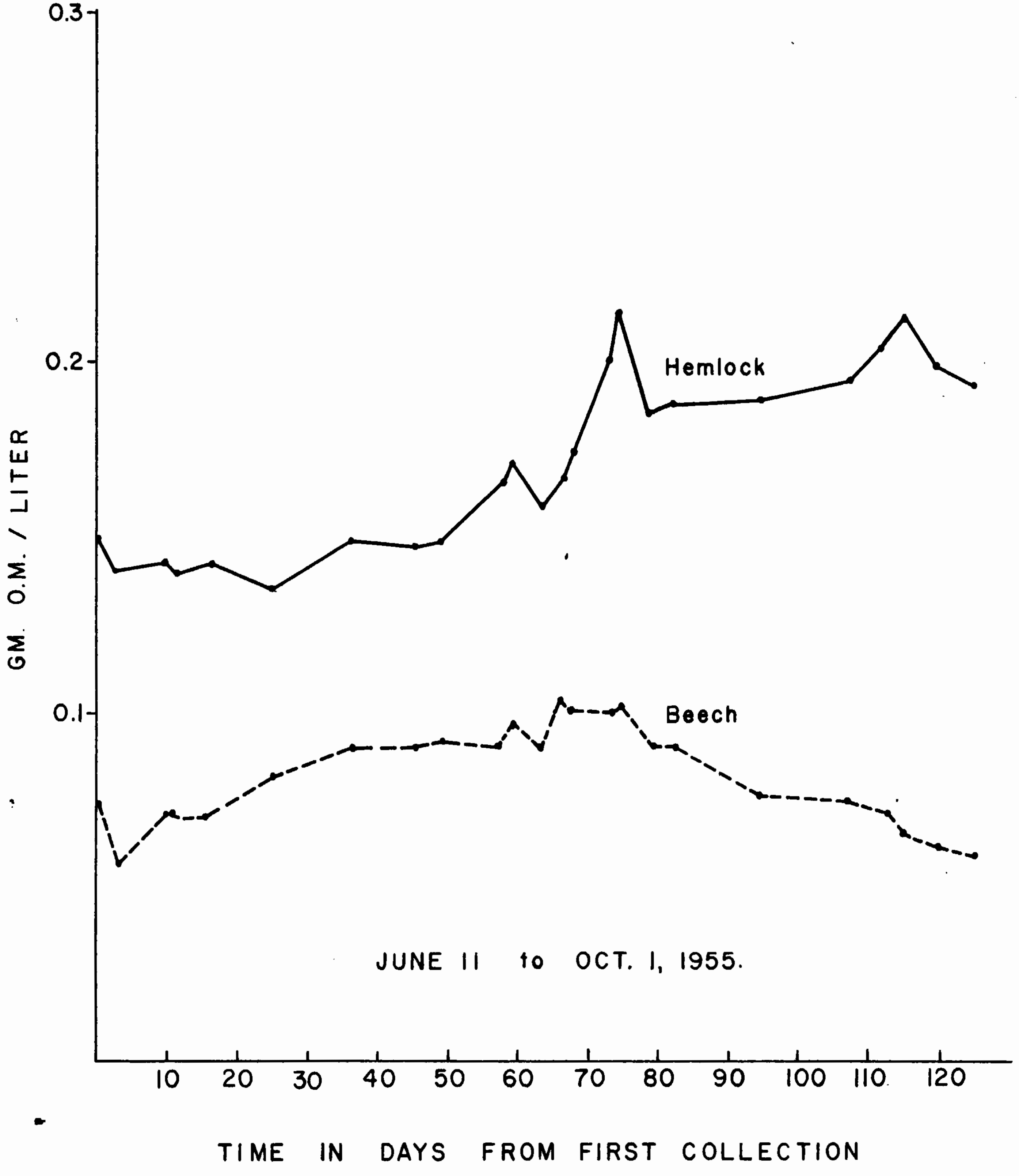


Figure 1. Organic matter content of beech and hemlock leachates

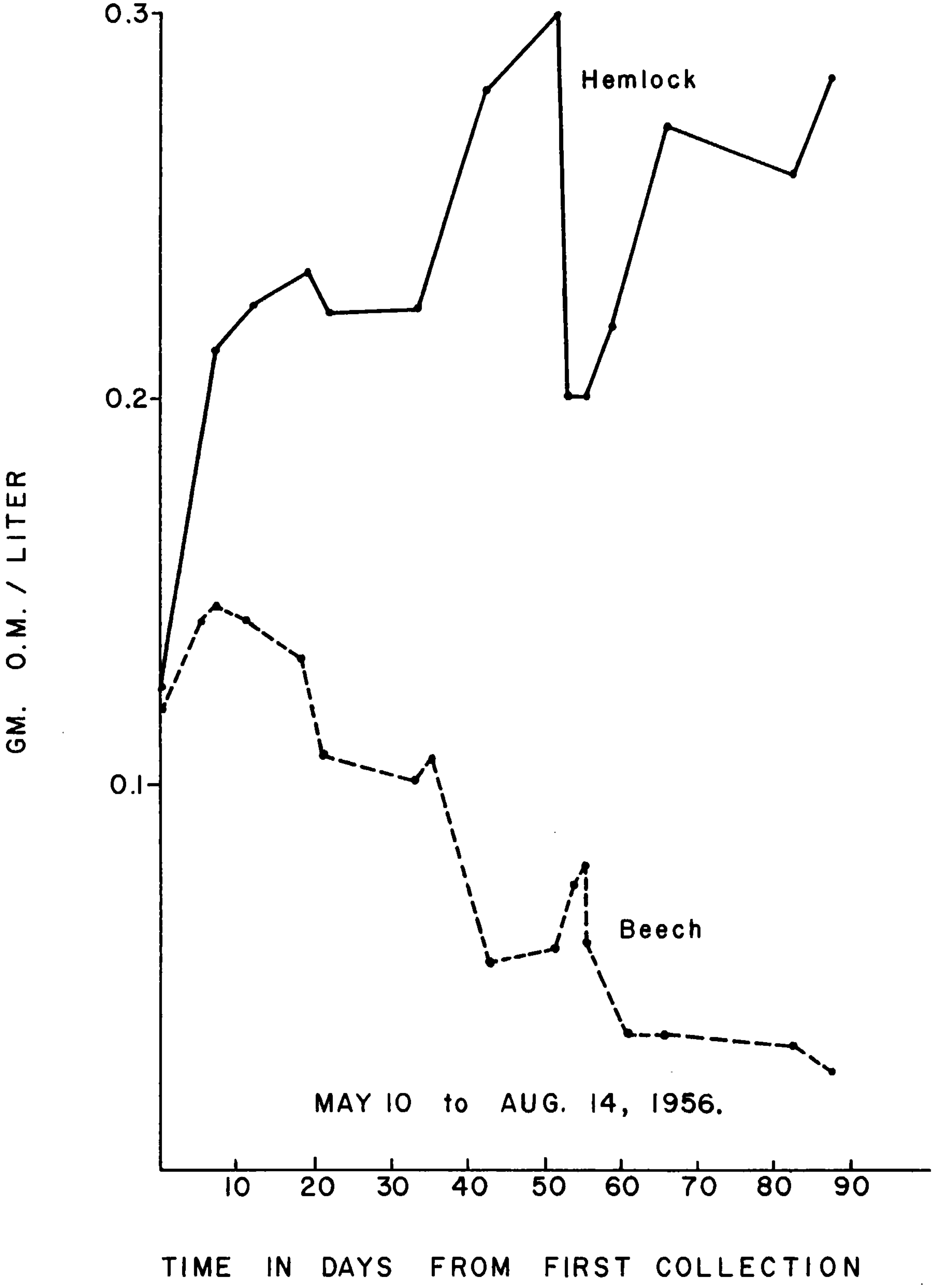


Figure 2. Organic matter content of beech and hemlock leachates

in leachates for individual trays varied considerably. In view of the well known variability of soils and of the fact that the A horizons leached have been disturbed, such variation is to be expected. It may be noted in this connection that differences in concentration of organic matter in leachates from individual trays have been maintained ever since the first leachate collection was obtained from these horizons. It is also possible that part of the difference between the trays was due to variation in the contribution to the leachates from the canopy.

The data for the inorganic solids' concentrations found in the two trays of leachate are given in Figures 3 and 4. The data presented there are, like the organic matter concentrations shown in Figures 1 and 2, mean values for the leachates obtained from four trays of each A horizon type. The quantities of inorganic solids present represent differences between total solids and organic matter. Comparison of Figures 3 and 4 with Figures 1 and 2 shows that the concentration of inorganic solids in these leachates is much more variable than the concentration of organic matter. This comparison also fails to reveal any definite seasonal differences in the concentration of inorganic material in the two types of leachates such as is suggested for the relative concentration of organic material. Although the concentration of beech and hemlock inorganic material often differs considerably for any given date, the trend of the values throughout the season is remarkably similar. No definite correlation of the average content of inorganic material in either type of leachate with either pH or rainfall volume could be established.

The only inorganic component of these leachates for which sufficient information has been obtained to warrant comment is iron. The

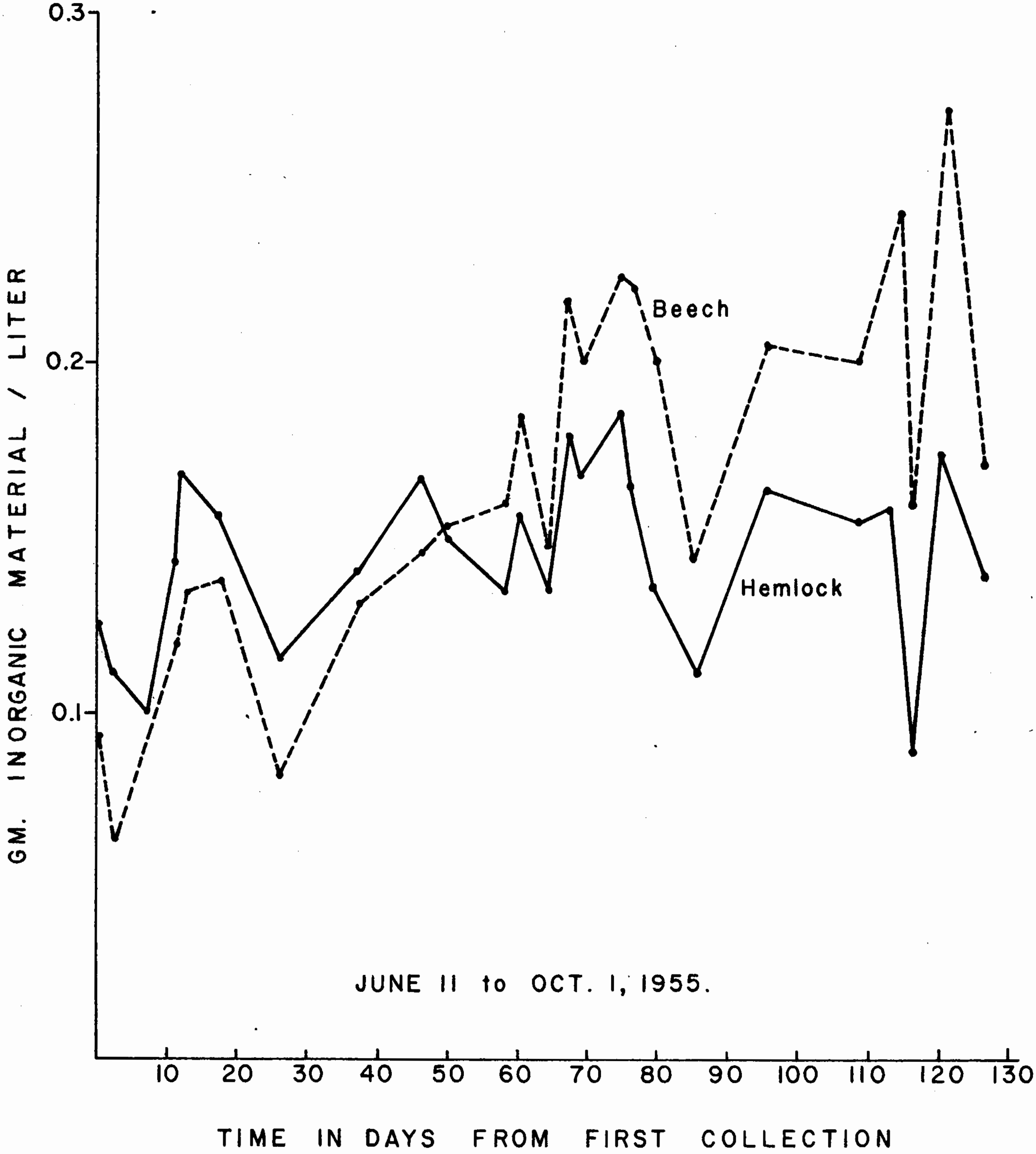


Figure 3. Inorganic material of beech and hemlock leachates

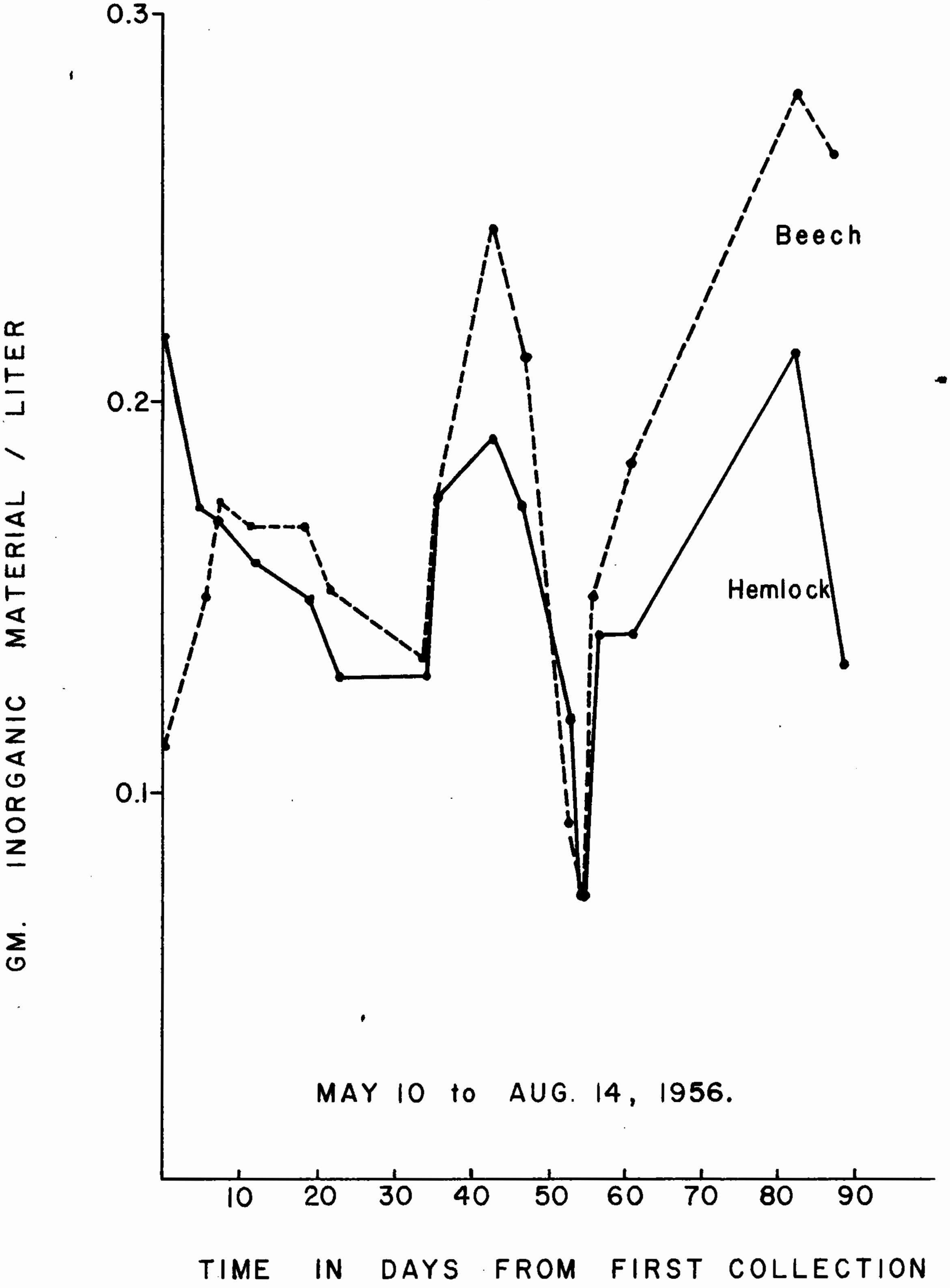


Figure 4. Inorganic material of beech and hemlock leachates

data available are presented in Table 1. Iron was determined on a composite sample of leachate obtained from four trays of each A horizon. Inspection of Table 1 shows that, as in the case of concentrations of total inorganic material, the beech leachates have a more variable concentration of iron than hemlock leachates. The relative constancy of the ratio of the concentrations of iron to those of organic matter throughout the period July 16 to August 31, 1955, and the marked similarity of these ratios for the two types of leachates suggests strongly that the removal of iron for these A horizons is closely related to the movement of organic matter. The iron-organic matter ratios for the 1956 season appear to be rising toward the levels found in 1955. The fact that, although essentially the same amount of iron per unit of organic matter was transported under both beech and hemlock, more organic matter moved downward under hemlock, indicates more active podzolization under the latter type of vegetation.

It may be noted that the ratios found for these leachates are of a similar order of magnitude to the values previously reported (21) for the iron uptake capacities of leaf extracts, leachates from decomposing leaves and canopy drip solutions.

Comparison of A Horizon Leachates with Leaf Leachates and Aqueous Leaf Extracts

The leachates described above are dilute and concentration of the organic matter in them facilitates the investigation of its nature. Beech and hemlock leachates which had been collected after rather large rainfalls late in the 1955 season were concentrated by vacuum evaporation at elevated temperatures (not exceeding 50 - 60°C.)

Table 1. Total iron content of beech and hemlock A horizon leachates.

Date collected	Iron content mgm. iron/liter		Iron/organic matter ratio mgm. iron/gm. organic matter	
	Beech	Hemlock	Beech	Hemlock
1955				
July 16	2.50	3.24	27.5	21.7
25	1.44	3.12	15.5	21.2
Aug. 8	1.80	4.00	18.5	23.2
12	1.98	3.54	21.9	22.3
15	1.77	3.00	17.5	18.0
16	2.72	3.60	22.2	23.5
23	2.28	4.08	22.4	18.8
27	1.83	4.02	20.0	21.3
31	1.82	3.90	20.2	21.1
			Mean 20.6 ± 2.5	Mean 21.2 ± 1.2
1956				
May 10	1.75	1.81	14.7	13.4
15	2.20	2.67	15.4	13.8
17	2.15	2.87	14.6	13.5
21	2.57	2.64	18.0	11.9
28	1.93	2.98	19.2	12.9
31		3.52		15.5
			Mean 16.4 ± 1.4	Mean 13.5 ± 0.7

DeLong and Schnitzer (21) in their studies on podzolization worked with aqueous leaf extracts and leachates obtained from natural rainfall passing through decomposing poplar leaves. They isolated a fraction from each, by precipitation in 80 per cent aqueous ethanol, which possessed the major part of the capacities of these solutions to react with iron. It was decided, as a starting point in the study of the nature of the organic matter in beech and hemlock A horizon leachates, to see if these solutions contained fractions similar to those isolated by DeLong and Schnitzer. Concentrated beech and hemlock leachates were subjected to the fractionation method of DeLong and Schnitzer using 80 per cent ethanol. It was found for leachates from both samples that although a large precipitate was formed in the first precipitation (67 to 75 per cent of the total organic matter), subsequent reprecipitations greatly diminished the size of the precipitate until after two or three reprecipitations no precipitate formed. It was also found that the fraction of the organic matter of these leachates which was separated from them by a single precipitation with 80 per cent ethanol had the same capacity (per gm. of organic matter) to retain iron in suspension as had the original leachates.

These leachates were produced by the passage of rainfall through beech and hemlock canopy and also through the O layer and the eluvial horizon of soil found under these canopies. On the other hand, the solutions investigated by DeLong and Schnitzer (21) were obtained either by extraction of poplar leaf powder in the laboratory or by passage of rainfall through beech canopy and then over decomposing poplar leaves without contact with either an O layer or soil from the eluvial horizon. The behaviour of the leachates presently under study in respect of precipitability with 80 per cent ethanol, of the relative capacities of the organic matter so precipitated and of the total organic matter of the leachates to retain iron in suspension, suggests strongly that the organic matter of such leachates differs markedly from the organic matter present in extracts and leachates obtained from poplar leaves under the conditions and with the methods used by DeLong and Schnitzer.

Electrophoretic Studies on Hemlock Leachates and B Horizon Extracts

Other than in the preliminary studies, only hemlock leachates were used from 1956 onward in the studies of the organic matter in A horizon leachates. The organic matter in these leachates was concentrated by iron precipitation followed by the extraction of iron with 8-quinolinol in chloroform.

The concentrated hemlock leachates obtained in September, 1956 were subjected to paper electrophoresis using several buffers at different pH levels. Under ultra violet light two electrophoretic components were distinguishable on the strip of filter paper when the pH of the buffer was 7.6 or greater. Both of these components moved toward the anode. The one nearer the anode fluoresced a light yellow under ultra violet light while the other which overlapped the first appeared as a dark band. When the pH of the buffer was 7.2, another component appeared near the origin which absorbed ultra violet light. At pH's 4.4 and 3.6 the band near the origin was more evident. At these lower pH's there was also a narrow band at the origin that fluoresced under ultra violet light.

Table 2 gives the results of a typical run at pH 4.4 with an acetate buffer. The results would indicate that the hemlock leachate contained at least four electrophoretic components. An electrophoretic run was also made with a leachate collected on June 3, 1957. The

Table 2. Electrophoretic run of hemlock leachate at pH 4.4 using an acetate buffer.

Component	Distance moved from origin toward anode in cm.	Appearance under		Stain with methylene blue	Stain with diazotized p-nitro aniline
		Ultra violet light	Visible light		
A	0	fluoresces	none	none	none
B	1.6	absorbs	brown	faintly	faintly
C	8.7	absorbs	brown	strongly	strongly
D	10.3	fluoresces	none	none	none

results were identical with those of the 1956 leachate. Two of these components stained with diazotized p-nitro aniline. The stain produced with p-nitro aniline is specific for aromatic compounds; therefore, it would appear that these two components contained aromatic compounds. The same two components also stained with methylene blue. This cationic dye has been used to stain acidic mucopolysaccharides (29). The fact that the two components stained with methylene blue probably indicates anionic groups were present.

Recent work (71) (62) on electrophoretic separation of organic matter extracted from soil suggests that in the solutions these investigators worked with there were two, and sometimes only one, electrophoretic components. Since there appear to be at least four electrophoretic components in the hemlock leachate, it was thought that it would be of interest to see how many components were present in a soil extract from the B horizon of a soil under hemlock canopy. The organic matter was extracted from a sample of the B horizon by mixing the soil with a hydrogen resin in water and shaking this mixture.

An electrophoretic run was made on the extract from the B horizon at a pH of 4.4 using an acetate buffer. The B horizon extract contained four electrophoretic components which, with the methods of detection used, were very similar to those found in the hemlock leachates. Any differences in these components seemed to be quantitative rather than qualitative. Components A and B were present in smaller amounts and component D in a larger amount in the B horizon extract than in the hemlock leachate. It would appear from these results that the B horizon under study contained electrophoretic components similar to those in leachates percolating through the A horizon.

Fractionation of the Hemlock Leachates and B Horizon Extracts

Since it was found in the electrophoretic examination of hemlock leachate and B horizon extract that both of these materials contained four detectable electrophoretic components, an attempt was made to isolate some of these components. The organic matter recovered from iron-precipitated leachates from the collection on June 3, 1957, and that extracted from the B horizon was found to be separable into four fractions. The initial step in the fractionation scheme used was the passage of the aqueous solution through a column of Dowex-50 cation exchange resin (hydrogen form) after which the effluent was neutralized with barium hydroxide solution. The precipitate obtained (fraction 1) was separated by centrifuging and washing with 80 per cent aqueous ethanol (v/v). The ethanolic washings were added to the supernatant from fraction 1 and the ethanol concentration of this mixture brought to 60 per cent (v/v) by addition of 95 per cent ethanol as required. This caused precipitation of fraction 2, fraction 3 remaining in solution.

Fraction 1 was sub-divided by first shaking with Dowex-50 cation exchange resin (hydrogen form) to remove barium and filtering to remove the resin. Addition of anhydrous sodium acetate to the solution so obtained to a concentration of 10 per cent (w/v), followed by adjustment to pH 6.5 with acetic acid or sodium hydroxide as required and addition of n-propyl alcohol to a concentration of 60 per cent (v/v), precipitated fraction 1a, fraction 1b remaining in solution. The organic matter in fraction 1b was found to be precipitable to the extent of about 90 per cent by addition of sufficient barium acetate to give a 5 per cent (w/v) solution.

This method, then, gave four fractions with both the hemlock leachate and the B horizon extract. In both cases the fractions were named 1a, 1b, 2 and 3.

Characterization of the Fractions Obtained from Hemlock Leachates and B Horizon Extracts

The first indication that a separation had been achieved was the colour of the fractions. Fractions 3, 2, and 1b of both the hemlock leachate and B horizon extracts were all much lighter in colour than fraction 1a even when the concentration of organic matter was the same. Fraction 1a appeared almost as dark as the original solution.

Electrophoresis was carried out on these fractions to see if the separation had actually isolated any electrophoretic component. Table 3 shows the results obtained by electrophoresis of three fractions of hemlock leachate and B horizon extract using an acetate buffer at a pH of 4.4. There is some evidence that the fractionation procedure was successful in separating different components of organic matter. Fraction 2 seems to be entirely composed of electrophoretic component 4. Fraction 1b is mostly composed of electrophoretic component 4, but has small amounts of components 1 and 3. Fraction 1a contains nearly all of the electrophoretic components 1, 2 and 3 with a trace of component 4. It was found that the trace of electrophoretic component 4 could be eliminated from fraction 1a by several reprecipitations with sodium acetate and n-propyl alcohol.

DeLong and Schnitzer (21) found that the fractions they had isolated from leaf extracts held considerably more iron in suspension

Table 3. Electrophoretic run of 3 fractions of hemlock leachate and B horizon extract.

Component	Distance moved from origin toward anode in cm.	Fractions in which the electrophoretic components are present		Appearance under		Stain with methylene blue	Stain with diazotized p-nitro aniline
		B horizon extract	Hemlock leachate	Ultra violet light	Visible light		
A	0	1a 1b*	1a 1b*	fluoresces	none	none	none
B	1.6	1a	1a	absorbs	brown	faintly	faintly
C	8.7	1a 1b*	1a 1b*	absorbs	brown	strongly	strongly
D	10.3	1a* 1b 2	1a* 1b 2	fluoresces	none	none	none

* in small amounts

per unit weight than did the original solution when measured by their saturation capacity method. In view of this it was thought that it would be of interest to see if any of the fractions isolated had any enhanced ability to hold iron in suspension. Saturation capacities were run on three of the fractions of the hemlock leachate. Fraction 3 was not included since no method was found to separate the organic matter from the alcohol.

Table 4 summarizes the information obtained up to this point concerning the composition and properties of the organic matter fractions obtained from the hemlock leachates and B horizon extracts. Inspection of the table shows that fraction 2 has a large saturation capacity compared to the other fractions. Fraction 1b has an intermediate saturation capacity while fraction 1a has a comparably small saturation capacity. There would seem to be some correlation between electrophoretic mobility and the amount of iron that the fractions are able to hold in suspension. This is not unreasonable in that both a high electrophoretic mobility toward the anode (the anode) and a large iron holding capacity would indicate a component rich in negative charges. The fact that electrophoretic component D did not stain with methylene blue might indicate a lack of anionic groups. However, it might also indicate that this component was composed of low molecular weight materials which could be washed off the paper in the process of staining. ?

If the amount of iron that a compound can hold in solution is any criterion of the ability of the compound to mobilize iron and transport it in soil, then fraction 2 would seem to be the most active in the podzolization process. That this fraction is a consistent part of the hemlock leachates is borne out by the fact that as a percentage of the total organic matter it varied less than 1 per cent on three collections made in September, 1956, May, 1957 and June, 1957.

Table 4. Composition and properties of the fraction of hemlock leachates and B horizon extracts.

Fraction	Electrophoretic components present		Organic matter content of fractions as a percentage of total organic matter		Saturation capacity of the leachate mg. Fe/gm. o.m.	Color at similar organic matter conc.	
	Leachate	Extract	Leachate	Extract		Leachate	Extract
1a	A B C D	A B C D	53	37	387	Dark brown	Dark brown
1b	A C D	A C D	17	26	785	Yellow	Yellow
2	D	D	24	32	1022	Yellow	Yellow
3			5.2	5.4		Light yellow	Light yellow

The fractions obtained above were characterized to some extent by analysis for metals, nitrogen, phosphorus, and carbohydrate, and by paper chromatography after acid hydrolysis.

Table 5 shows in the first column the per cent of the total organic matter found in each fraction and in subsequent columns the percentages of nitrogen and of carbohydrate in the organic matter of the individual fractions of the hemlock leachate and B horizon extract. Fractions 2 and 1a of the B horizon extract make up a larger proportion of the total organic matter and fraction 1b makes up a smaller proportion of the total organic matter than do similar fractions of the hemlock leachate. There was some indication of this in the results from electrophoresis.

Except for fraction 3, the percentage of nitrogen is fairly uniform throughout the fractions and differences between the hemlock leachate and the B horizon extract are small. The result for fraction 3 of the hemlock leachate was not recorded since nitrate was present and was expected to interfere in the estimation of organic nitrogen. The per cent of carbohydrate varies in the fractions much more than does nitrogen. The carbohydrate content of the hemlock leachate is somewhat higher than the B horizon extract, but the fractions of each show a similar trend in per cent carbohydrate. In both hemlock leachate and B horizon extract, carbohydrate is surprisingly low being 2.2 and 1.9 per cent, respectively and therefore contributes very little to the total organic matter. These results are in agreement with those of MacLean and DeLong (45) who found that the carbohydrate content of pine A horizon leachates was low (4.8 per cent) as compared to leachates passing through pine litter (17.9 to 28.6 per cent). In the present

Table 5. Percentage of the total organic matter in fractions of hemlock leachates and B horizon extracts and nitrogen and carbohydrate as a percentage of the organic matter in the fractions.

Fraction		% of total organic matter	% Nitrogen	% Carbohydrate as glucose
1a	L	53.5	.80	1.2
	E	36.9	.62	1.0
1b	L	17.1	.75	.5
	E	26.1	.70	.9
2	L	24.3	.94	4.0
	E	31.6	.90	2.5
3	L	5.2	-	10.4
	E	5.4	2.12	6.8

L = Leachate, E = B horizon extract

Table 6. Phosphorus, iron and aluminum content of fractions of hemlock leachates and B horizon extracts.

Fraction	mgm./gm. of organic matter		
	Phosphorus	Iron	Aluminum
Leachate			
1a	0.2	0.5	1.8
1b	6.3	0.3	3.0
2	12.8	0.4	1.9
3	1.2	2.8	5.2
B extract			
1a	1.1	12.5	5.7
1b	1.5	0.2	1.7
2	0.3	3.7	10.5
3	0.7	3.3	10.7

instance, the percentage of carbohydrate in the whole hemlock leachate may well have been higher than that found for the organic matter precipitated by iron. The organic matter which was not precipitated by iron (10 per cent) may have had a high carbohydrate content.

Qualitative analysis for sugars was run on the acid hydrolyzate of the hemlock leachate and B horizon extract by means of paper partition chromatography. Five sugars were detected in both hydrolyzates in very small amounts and tentatively identified as galactose, glucose, arabinose, xylose and rhamnose.

The analyses of the fractions of phosphorus, iron and aluminum are given in Table 6. The leachate contains considerably more phosphorus than does the B horizon extract. Ninety-one and sixty-five per cent of the phosphorus in the leachate and B horizon, respectively, was inorganic. Most of the phosphorus in the leachate (71 per cent) appears in fraction 2 and nearly all the rest is in fraction 1b. The fractions of the B horizon extracts are lower and more uniform in phosphorus than the leachate. Fraction 2 of the B horizon extract, in contrast to fraction 2 of the leachate, contains the smallest percentage of phosphorus.

The iron and aluminum contents of the leachate fractions are low. This means that most of the iron and aluminum could be removed from these solutions with 8-quinolinol extractions combined with treatment with a hydrogen resin. The iron and aluminum contents of the B horizon extract fractions are generally considerably higher than those of the leachate. The B horizon extracts had not been extracted with 8-quinolinol and this possibly explains the difference. Fraction 1b of the B horizon extract is comparatively very low in iron and aluminum. This might be due to the fact that in the separation this fraction had been treated twice with a hydrogen resin, whereas fractions 1a and 2 had only been treated once and fraction 3 not at all. In all fractions of the leachates and B horizon extract the remaining traces of aluminum seemed to be more difficult to remove than iron.

Paper Chromatography

Amino acids contained in the 6N HCl hydrolyzates of the hemlock leachate and B horizon extract were separated and tentatively identified by means of paper partition chromatography. The hydrolyzates of the whole leachate and B horizon extract were run on two dimensional chromatograms. The chromatographic patterns of the two solutions were identical. The amino acids tentatively identified were: cysteic acid, glutamic acid, serine, glycine, lysine, arginine, aspartic acid, threonine, alanine, leucine and/or isoleucine and methionine and/or valine. One dimensional chromatograms were run with 6N HCl hydrolyzates of fractions 1a, 1b and 2 of the hemlock leachate and B horizon extract and of the whole solutions. The patterns of spots on the chromatogram when stained with ninhydrin were identical for all fractions of both the hemlock leachate and B horizon extract and also of the whole solutions. It would seem that the amino acid composition was uniform throughout the fractions separated and that the amino acid composition of the hemlock leachate was similar to that of the B horizon extract.

For detection and separation of compounds other than amino acids, one dimensional chromatograms were run with the hydrolyzates of fractions 1a, 1b and 2 of the hemlock leachate and B horizon extract that were prepared for amino acid analysis. The solvent used was n-butanol - acetic acid-water (4:1:2.2 v/v). Table 7 gives the results

Table 7. R_f values with the solvent n-butanol - acetic acid - water (4:1:2.2 v/v) of phenolic materials obtained from an acid hydrolyzate of fractions of a hemlock leachate and B horizon extract and their appearance under ultra violet light.

H-2		H-1a		E-2		E-1a	
fluorescent	not fluorescent	fluorescent	not fluorescent	fluorescent	not fluorescent	fluorescent	not fluorescent
.04		.04		.05	.10	.04	.08
.12			1.5	.12	.15	.12	.14
.22		.22		.22		.22	
.32		.33		.34		.36	
Trailing .32 .90	.78			Trailing .34 .90		Faint trailing .36 .90	

H - hemlock leachate

E - B horizon extract

of runs with fractions 1a and 2 of each type of solution. The results for the 1b fractions are not included since they were very similar to those for the No. 2 fractions. The spots detected stained with both ferric chloride/ferricyanide and diazotized p-nitro aniline. This indicates that they were phenolic in nature. Some of the spots also fluoresced under ultra violet light. Inspection of Table 7 shows that fractions 2 and 1a of the hemlock leachate differ in some components. A non-fluorescent spot appeared in fraction 1a which was not present in fraction 2. Fraction 2 had a fluorescent spot at R_f value .12, a non-fluorescent spot at R_f value .78 and a fluorescent streak from R_f value .33 to .90 which fraction 1a did not reveal.

There was not as much difference between fractions 1a and 2 of the B horizon extract as there was between similar fractions of the leachate. The only noticeable difference between the two fractions was that the fluorescent trailing in fraction 1a from R_f value .36 to R_f value .90 was much less than in fraction 2. In all the chromatograms of both types of solutions, a great deal of material did not move out of the origin. The material at the origin also stained strongly with both ferric chloride/ferricyanide and diazotized p-nitro aniline. The hydrolysis of all of the fractions of both types of solutions was incomplete as there was a large amount of insoluble material at the end of the heating period. Except for non-fluorescent compounds, the chromatograms of the No. 2 fractions of hemlock leachate and B horizon extract were very similar.

Attempts were made to identify some of the phenolic compounds separated by paper chromatography; however, no success was attained. Several solvents were used but no spots were obtained that had colour reactions with phenolic stains, fluorescent properties under ultra violet light, or R_f values that consistently corresponded to values found in the literature. Different normalities of acid and times of hydrolysis were tried but were of no avail. This method of attack was therefore dropped.

Studies on the Products of Potassium Hydroxide Fusion with Hemlock Leachates and B Horizon Extracts

Since acid hydrolysis of the hemlock leachate and B horizon extract gave evidence that phenolic materials were present and because the products formed proved difficult to identify, it was decided to use the method of KOH fusion to study the "building blocks" of these materials. Initially only the hemlock leachate, collected on June 3, 1957, was studied. The leachate, after evaporation to dryness on a steam bath, was fused with KOH in air. The fused mass was then dissolved in water and acidified with HCl. The acid solution was extracted with ether. The ether solution was then evaporated to dryness on a steam bath. The amount of material recovered in the ether extract varied from 15 to 35 per cent of the original material depending upon the time of fusion. As the time of fusion was increased the amount of material recovered was decreased.

The fused material was taken up in a small volume of ether and the solution was studied by means of paper partition chromatography. Several solvents and stains were used with varying success. It was found that the solvent isopropyl alcohol-aqueous ammonia-water (8:1:1 v/v), used by Armstrong (2) in the study of phenolic acids, was the most satisfactory. With the other solvents, some of the compounds either ran together or were not clearly separable. By use of this solvent and diazotized stains it was possible to detect five well defined spots on the chromatogram. Tentative identification of the compounds was achieved by comparing the colour reactions of the spots after staining and their R_f values with those found in the literature. Authentic samples of these five compounds were obtained and run on the same chromatogram as the unknown. Again several solvents were used and in all cases the unknown compounds were found to have the same R_f values and the same colour reactions with the diazotized stains as the known compounds. Table 8 lists the R_f values, the colour reactions with the diazotized stains and the tentative identification of the unknowns found in a typical run of the fused products of the hemlock leachate organic matter. The fusion time used gave approximately 35 per cent recovery of the organic matter. Inspection of the table shows that unknowns 1 to 4 have been tentatively identified as phenolic acids and unknown 5 as a phenol.

The tentative identifications of unknown materials made in the manner outlined above is usually correct. However, it is not a positive identification. For positive identification, isolation of the compound is necessary. If the compound is isolated and has the same melting point as the known compound, and its melting point is not changed by mixing with an authentic compound, then positive identification is usually considered to be proven.

It was desirable, then, to isolate the phenolic compounds in quantities such that melting points could be obtained. A large quantity of the hemlock leachate was fused with potassium hydroxide and extracted with ether as had been done previously. This time, however, phenolic acids were separated from non-acidic phenols by washing the ether solution with an aqueous solution of sodium bicarbonate, the phenolic acids being

Table 8. Phenolic materials obtained from a potassium hydroxide fusion of the organic matter in a hemlock leachate.

Unknown	R_f in the solvent Ipr. NH_3 - H_2O*	Colour reaction		Tentatively identified as
		Dz PNA**	Dz SA***	
1	.24	brown	brown	3,5-dihydroxy benzoic acid
2	.29	red	yellow	p-hydroxy benzoic acid
3	.35	dark brown	dark brown	2,4-dihydroxy benzoic acid
4	.44	red	yellow	m-hydroxy benzoic acid
5	.82	brown	none	m-dihydroxy benzene

* Ipr. NH_3 - H_2O - Isopropanol - aqueous ammonia - water (8:1:1 v/v)

** Dz PNA - diazotized p-nitro aniline

*** Dz SA - diazotized sulfanilic acid

extracted into the sodium bicarbonate solution leaving the non-acidic phenols in the ether phase. The phenolic acids were recovered by acidifying the sodium bicarbonate and extracting again with ether. The solution containing the phenolic acids was evaporated to dryness. The residue was taken up in a small quantity of the solvent isopropyl alcohol - aqueous ammonia - water (8:1:1 v/v), and the acids were separated on a cellulose column using isopropyl alcohol - aqueous ammonia - water (8:1:1 v/v) as the developing solvent. The compounds after a further purification process were white in colour and crystalline in nature. Unsuccessful attempts were made to purify the non-acidic phenolic material. The material always remained a dark brown colour. It melted slightly above room temperature. Melting points and mixed melting points were done on the separated phenolic acids.

The unknown compound thought to be p-hydroxy benzoic acid melted at 209 - 211°C. The authentic compound also melted at 209 - 211°C. Mixing the authentic compound with the unknown compound did not depress the melting point. The unknown compound thought to be 3,5-dihydroxy benzoic acid melted at 230 - 234°C. The authentic compound melted at 232 - 235°C. The unknown and authentic compounds mixed together melted at 230 - 234°C which was the same as the unknown. The unknown thought to be m-hydroxy benzoic acid melted at 197 - 201°C. The authentic compound melted sharply at 199°C. The unknown and authentic compounds mixed together melted at 197 - 201°C. It was therefore assumed that these compounds had been positively identified. The compound thought to be 2,4 - dihydroxy benzoic acid occurred in only small amounts and no melting point was obtained.

Since it was possible to identify positively three of the four aromatic acids, it was permissable to do a quantitative determination. Pridham (54) has developed a method for the quantitative determination of phenolic glycosides and aglycones. This method consists of separating the phenolic compounds by paper partition chromatography, coupling them on paper with diazotized p-nitro aniline, and eluting the spots with a solution of potassium hydroxide in alcohol. The concentration of the coloured solutions are then determined quantitatively using a spectrophotometer. This method was used for the quantitative determination of the four phenolic acids. Positive identification had not been obtained for 2,4 - dihydroxy benzoic acid; nevertheless, it was assumed that the tentative identification was correct. Standard curves were run with authentic compounds after they had been chromatographed with isopropyl alcohol - aqueous ammonia - water (8:1:1 v/v) as a developing solvent, stained with diazotized p-nitro aniline and eluted with potassium hydroxide in alcohol. The intensity of the colour of the resulting solution bore a linear relationship, after subtraction of blanks, with the weight of the compound. Table 9 shows the amount of the phenolic acids obtained on fusion with potassium hydroxide as a percentage of the amount of the original organic matter.

It was found earlier that organic matter extracted from the B horizon of the podzol soil studied resembled in many ways the hemlock leachate. Therefore, it was thought to be of interest to see if this material contained the same phenolic acids after fusion with potassium hydroxide as did the hemlock leachate. The organic matter extracted from the B horizon was fused with potassium hydroxide and treated in the

Table 9. Phenolic acids as a percentage of the original organic matter in a hemlock leachate and B horizon extract.

	Per cent of the original organic matter	
Phenolic Acid	Hemlock leachate	B horizon extract
m-hydroxy benzoic acid	2.8	3.0
p-hydroxy benzoic acid	2.1	2.3
3,5-dihydroxy benzoic acid	0.8	1.0
2,4-dihydroxy benzoic acid	0.5	0.6

same manner as the hemlock leachate. The amount of material which was recovered in the ether was of the same order of magnitude as that obtained with the hemlock leachate. Chromatographic studies revealed that the organic matter from the B horizon extract after fusion with potassium hydroxide contained the same five phenolic compounds as did the hemlock leachate. Many solvents were used and in all cases the chromatographic patterns of the compounds were identical with the compounds obtained from the hemlock leachate. This was taken as evidence that the compounds obtained from the fusion of the B horizon extract were identical to those obtained from the hemlock leachate. The phenolic acids obtained from the B horizon extract were not isolated; therefore, melting points were not determined. Assuming that the materials were the same as those isolated from the hemlock leachate, a quantitative determination was made on the four phenolic acids. The results are given in table 9. It is evident that the recovery of the four phenolic acids from the fused hemlock leachate was approximately equal to the recovery from the B horizon extract.

It has been shown previously by means of chemical analysis, electrophoresis, iron saturation capacities and acid hydrolysis that there were some differences in the fractions obtained by the separation procedure outlined earlier. Fractions 1a, 1b and 2 of both hemlock leachate and B horizon extract were fused with KOH to ascertain if the products produced were the same for all fractions. The resulting material after extraction with ether was subjected to chromatography using several solvents. In all fractions the detectable products of KOH fusion were identical to those obtained from the whole leachate and B horizon extract. Quantitative analysis was not run, but it was evident from the size and intensity of the spots after staining that the amounts of phenolic acids obtained per unit of organic matter were approximately equal for all six fractions as that obtained from similar amounts of the organic matter from the whole leachate and B horizon extract.

A large portion of the organic matter in both the hemlock leachate and the B horizon extract (up to 35 per cent in a short fusion) was recovered in ether after the potassium hydroxide fusion. The ether solutions had been washed with a solution of $NaHCO_3$, a procedure which normally separates phenolic acids from non-acidic phenolic materials. In both the hemlock leachate and B horizon extract, 90 to 92 per cent of the organic matter was extracted into the $NaHCO_3$ which would indicate that it was acidic in nature. It is not surprising that the material was acidic since the original material before fusion had an equivalent weight when titrated in the acid form with sodium hydroxide, of the order of 160. It is possible that this low equivalent weight is due partly to inorganic material; however, fraction 2 which might be expected to be in a purer state had a lower equivalent weight, namely 130.

Although the amount of material recovered in ether from the KOH fusion was relatively high, only a small percentage of this material was such that it could be easily identified. The amounts reported are minimal since it is quite possible that these compounds are destroyed almost as quickly as they are formed. This was borne out by the fact that if the fusion was allowed to go just a little too far, the amount of material recovered in the ether, and the amount identified, were both greatly reduced. It is impossible to say from what materials these phenolic acids were derived. Many phenolic compounds, when subjected to KOH fusion, will break down to yield phenolic acids such as these. It was found by the author that gallic acid when oxidized by raising its pH to 9.0 with sodium hydroxide and shaking in air for a few days, yielded, after potassium hydroxide fusion, the same four phenolic acids in small amounts as did the leachate and B horizon extract. There may be many phenolic compounds in the A horizon of this soil which are capable of producing simple phenolic acids on fusion with potassium hydroxide.

An important aspect of this potassium hydroxide fusion study was that the hemlock leachate and the B horizon extract both yielded the same phenolic acids in approximately the same amounts. This is further evidence that these materials are similar in nature.

Table 10. Organic constituents of the hemlock leachate.

Constituent	Per cent of total organic matter
Crude protein	5.2
Carbohydrate	2.2
Isolated phenolic acids	6.2
TOTAL	13.6

Table 10 summarizes the results obtained on the organic matter composition of the hemlock leachate. It can be seen that the major portion of the organic matter is unaccounted for. The results from the potassium hydroxide fusion of the organic matter would indicate that the phenolic materials probably account for a much larger portion of the organic matter than the table would indicate.

The Study of Organic Matter in Canopy Drip Solutions

It has been shown by MacLean and DeLong (45) and DeLong and Schnitzer (21) that canopy drip solution, obtained by collecting rain that has passed through a forest canopy, contained appreciable amounts of organic matter. It was thought that the next logical step in the study of the organic matter in leachates would be to study organic matter found in the hemlock canopy drip. Comparison of the quantity of organic matter found in the hemlock leachate with the amounts found in canopy drip solutions showed that for four rainfalls between June 16 and July 17, 1958 the canopy drip contained from 16.0 to 32.4 per cent of the amount of organic matter found in leachates collected at the same time.

The iron saturation capacities of these canopy drip solutions were measured. It was found that the saturation capacities of the canopy drip solutions were about one-half of those of the leachates. These hemlock canopy drip solutions, therefore, cannot, under the experimental conditions used, hold as much iron in suspension as does the hemlock leachate. If this material percolates through the A horizon of the soil without any alterations, then the organic matter of the leachate which originates in the Ao horizon must have a much greater capacity to hold iron in suspension than does the canopy drip solution.

Iron and aluminum determinations were made on the canopy drip solutions. Only trace amounts of each were found.

A large volume of the canopy drip solution was evaporated down on a steam bath and fused with potassium hydroxide. Chromatograms were run with the fused material using the solvent isopropyl alcohol-aqueous ammonia-water (8:1:1 v/v). The chromatograms, after staining with the phenol stains, resembled those run with the fusion products of the hemlock leachate. The same four phenolic acids were present along with the spot that was tentatively identified as m-dihydroxy benzene. Quantitative analyses were run on the four acids using the Pridham (54) method. Table 11 gives the results of these determinations. Inspection of the table shows that these acids were not present in the fusion products of the canopy drip solution in as large quantities as they were in the hemlock leachate. The fact that they were present, however, shows that the canopy drip contributes phenolic materials to the forest floor. None of

Table 11. Phenolic acids as a percentage of the original organic matter in a hemlock canopy drip solution.

Phenolic Acid	Per cent of the original organic matter
m-hydroxy benzoic acid	0.9
p-hydroxy benzoic acid	0.8
3,5-dihydroxy benzoic acid	trace
2,4-dihydroxy benzoic acid	trace

the eleven phenolic materials found later in a hemlock needle extract was detected in the canopy drip solution. No new light was shed on the nature of the organic matter in hemlock leachates from this brief study of canopy drip solutions.

Studies on Hemlock Needle Extracts

Much work has been done with leaf extracts in the investigation of the podzolization process (21) (55) (12). Some of the workers have based theories concerning nature of the podzolization process on results from studies of aqueous leaf extracts of picked and fallen leaves. There is scant information on the compounds present in these aqueous leaf extracts. DeLong and Schnitzer (21) isolated material from a leaf extract that resembled an acidic polysaccaride. Raudnitz (55) found material in several leaves that he believed was a humic acid. Bloomfield (12) claimed that a large portion of the organic matter in the leaf extracts that he studied was phenolic in nature. He did not, however, report the occurrence of definite compounds of phenolic type. Swain (64) has suggested that in leaf litter there are two types of phenols, (a) those which will precipitate protein and are non-accessible in the litter and (b) the soluble compounds such as chlorogenic acid which will solubilize iron. Chlorogenic acid is known to have a wide distribution in the plant kingdom. Gibbs (26), who is making an extensive study of the chemical composition of plants in relation to their taxonomic classification, found it so generally distributed as to be of little use to him in this connection. For these various reasons it seemed desirable to study an aqueous extract of hemlock needles, collected from the canopy under which the hemlock leachates and canopy drip solutions were obtained, in order to look for the occurrence of such compounds as chlorogenic and/or their degradation products.

The hemlock needle extracts obtained were acid in nature, having a pH of 3.9 to 4.0. Approximately 25 per cent of the leaf material was accounted for as organic matter in the extract.

The hemlock needle extract was studied by means of paper partition chromatography using several solvents. The solvent n-butanol - acetic acid - water (4:1:2.2 v/v) gave the best separation of spots of any of the solvents. Table 12 gives the results of a typical chromatographic run of the hemlock needle extract with the solvent n-butanol - acetic acid - water (4:1:2.2 v/v) and the results of tests with several stains and of observations under ultra violet light. A dried chromatogram was stained with ferric chloride/ferricyanide which is a general

Table 12. Chromatographic run of a hemlock needle extract using the solvent n-butanol - acetic acid - water (4:1:2.2 v/v).

		- Test -					
Unknown number	R_f value	Ferric chloride ferricyanide	Ammonia vapor	Hoepfner	Ferric chloride	diazotized p-nitro aniline	ultra violet light
		- Colour -					
		blue	yellow	pink	green	orange	yellow
1	.13	+					
2	.19	++				+	
3	.25	+++			+	++	
4	.28		+	+			+
5	.30	++				+	
6	.34	+					
7	.39	+++	+	+	+	++	+
8	.43	+				+	
9	.55	++++++++	++++++	++++++	++++	+++++	++++
10	.60	+				+	
11	.73	+				+	
12	.87	+				+	

+ signifies a positive test and gives an indication of the intensity.

test for polyphenols. Eleven spots of varying intensities and sizes gave a blue colour with this stain. One spot, at R_f value .55, stained particularly well. The intensity of the stain and the size of the spot was approximately equal to the other ten spots combined. Another chromatogram was stained with diazotized p-nitro aniline which is specific for aromatic materials. Nine out of the eleven above compounds stained orange with this stain. This stain was not as sensitive as ferric chloride/ferricyanide. The two spots that did not stain with this reagent were very faint even with ferric chloride/ferricyanide. Another chromatogram was stained with ferric chloride. Only three spots, the ones that stained most strongly with the two phenolic stains, gave a green stain. It is possible that the other spots would have stained had they been present in larger amounts. The green colour with this test is indicative of the catechol grouping; that is, two adjacent hydroxy groups on a benzene ring. A chromatogram was tested for anthoxanthins by holding it over ammonia. Three yellow spots appeared on the chromatogram. One of these spots had not been detected before. The other two had stained strongly with the phenolic stains. Another chromatogram was tested for catechins and leuco-anthocyanins but no positive results were obtained. This result is in agreement with Williams (70) who states that the similar phenolic materials in leaves are mostly flavones and hydroxy cinnamic acids while catechins and leuco-anthocyanins are less abundant. The Hoepfner test for chlorogenic acid was next used on the chromatogram. The spot at R_f value .55 stained a strong pink. The other two spots that gave a positive test for anthoxanthins, stained a weak pink. These three spots were also the only ones that fluoresced under ultra violet light. It is thought that these spots probably represent related hydroxy cinnamic acid derivatives.

The spot at R_f value .55 was found to resemble chlorogenic acid in many of its properties. A sample of authentic chlorogenic acid was obtained and tests were run to compare this compound with the unknown. Chromatograms were run where the authentic chlorogenic acid was placed on the same chromatogram as the unknown material. Several solvents were used and in all cases the R_f value of the chlorogenic acid was the same as that of the unknown substance. The following tests were made on both the authentic chlorogenic acid and the unknown material after they had been spotted on filter paper.

1. Three tests for phenolic materials including ferric chloride/ferricyanide, diazotized p-nitro aniline and diazotized sulfanilic acid,
2. ferric chloride, which is a test for the catechol group,
3. the colour developed when held over ammonia vapours,
4. the fluorescent colour under ultra violet light before and after holding over ammonia vapours,
5. the colour developed when dipped into a solution of aluminum chloride and then held over ammonia vapours.

The results were identical for both substances which would indicate that the unknown was chlorogenic acid. This is not a positive identification, but there is a strong probability that it is correct.

A semi-quantitative determination of the amount of chlorogenic acid in the hemlock needle extract was made. This was accomplished by chromatographing known amounts of chlorogenic acid and known volumes of the extract on the same chromatogram. This procedure was repeated until the size and intensity of the chlorogenic acid spot when stained with several stains corresponded to that of the unknown. It was found that chlorogenic acid accounted for about 20 per cent of the organic matter in the hemlock needle extract. This would mean that the hemlock needles contained at least 5 per cent chlorogenic acid.

The hemlock leachate was chromatographed using the n-butanol - acetic acid - water solvent (4:1:2.2 v/v) to see if it contained any of the phenolic materials that were present in the hemlock needle extract. None of the eleven phenolic materials that were present in the hemlock needle extract was detected on the chromatogram. Nearly all of the material in the leachate remained at the origin of the chromatogram. A small apron of material that fluoresced under ultra violet light moved out of the origin. Other than this, nothing was detected on the chromatogram with any of the stains.

The hemlock leachate had been concentrated by precipitation with ferric nitrate. It is possible that this method of concentration might have altered the properties of any phenolic materials originally present in the leachate. A fresh collection of hemlock leachate was concentrated by evaporation on a steam bath. This concentrated material was then chromatographed using the solvent n-butanol - acetic acid - water (4:1:2.2 v/v). The resulting chromatogram was identical with the chromatogram of the hemlock leachate precipitated with iron. This would indicate, if the heating did not interfere, that the fresh leachate did not contain, in an unaltered form, any of the phenolic materials that were present in the hemlock needle extract.

The addition of ferric nitrate to a solution containing chlorogenic acid produces a green colour even when the concentration of the chlorogenic acid is relatively low. The leachate has never shown this green colour with the addition of ferric nitrate. This is further proof that chlorogenic acid was not present in the fresh leachate.

As mentioned previously, none of the eleven phenolic materials found in the hemlock needle extract was detected in the concentrated canopy drip solutions.

It was observed that the hemlock needle extract, after having been allowed to stand in air for several days, turned a darker colour even when chloroform was present. An experiment was initiated to see if the passage of air through the hemlock needle extract had any effect on the phenolic materials present. Ordinary air was sucked through cotton wool into the solution which was contained in a suction flask. Chloroform was added to the extract in an attempt to prevent microbial activity. Every few days chromatograms were run using this material. The solution became darker with the passage of time but even after ten days the chromatograms were similar to those run with the freshly prepared extract. The air was allowed to pass through the solution for another twenty days without any further testing. At the end of the twenty days the solution had turned a dark brown. A chromatogram was run with this material and this time the phenolic materials were not evident; in fact, the chromatograms resembled a chromatogram run with the iron precipitated leachate. It was not proven that the addition of chloroform to the solution of hemlock needle extract prevented all microbial activity. There was also no attempt made to stop phenoloxidase activity. Therefore, it is quite possible that there were more reactions going on than just the oxidation of materials by air.

The fresh hemlock needle extract was next fused with potassium hydroxide, extracted with ether and chromatographed in a similar manner as the hemlock leachate. The results indicated that the same four phenolic acids were present that had been identified in the fused hemlock leachate, namely, p-hydroxy benzoic acid, m-hydroxy benzoic acid, 3,5-dihydroxy benzoic acid and 2,4-dihydroxy benzoic acid. The phenolic compound tentatively identified as m-dihydroxy benzene was also present. There was also a very large spot present with a low R_f value.

The products of the fused hemlock needle extract were chromatographed in several solvents. In every case the large phenolic spot ran with the same R_f value and stained with phenolic stains as did protocatechuic acid. A small amount of chlorogenic acid was fused with KOH. Chromatographing the products of the fusion in several solvents and staining with the phenolic stains revealed one spot that corresponded to protocatechuic acid. It was assumed, therefore, that most of the protocatechuic acid found in the fused hemlock needle extract came from chlorogenic acid.

Quantitative determinations were made of the contents, in the extract from fresh hemlock needles, of the four phenolic acids also found in hemlock leachate after fusion. The Pridham (54) method was used. An estimate was also made of the amount of protocatechuic in the fused hemlock needle extract in a similar manner to that used for the estimation of chlorogenic acid in the needle extract. Table 13 shows the results of these analyses. Inspection of the table shows that protocatechuic acid was the only compound present in an appreciable

Table 13. Phenolic acids as a percentage of the original organic matter in hemlock needle extract.

Phenolic Acid	Per cent of the original organic matter
3,4-dihydroxy benzoic acid	8.0
m-hydroxy benzoic acid	0.8
p-hydroxy benzoic acid	0.5
3,5-dihydroxy benzoic acid	trace
2,4-dihydroxy benzoic acid	trace

amount. The other four acids did not represent as large a portion of the original organic matter as they did in the fused hemlock leachate.

The hemlock needle extract which had had air passed through it for thirty days was fused with potassium hydroxide. The products of potassium hydroxide fusion were chromatographed with ispropyl alcohol - aqueous ammonia - water (8:1:1 v/v). The resulting chromatogram was similar to chromatograms run with the fused freshly prepared hemlock needle extract with the exception that it had no large spot corresponding to protocatechuic acid. The chromatogram, in fact, was almost identical to a chromatogram run with the fused hemlock leachate. Apparently compounds such as chlorogenic acid had been altered sufficiently by the passage of air (or other factors) so that they no longer yielded protocatechuic acid on potassium hydroxide fusion. It would appear that the compounds found in hemlock needle extracts are relatively unstable. It is not surprising, then, that these compounds were not found in the hemlock leachate. Steele (61) has pointed out that since chlorogenic acid contains the ortho-dihydroxy grouping it is oxidizable to a quinone. She also stated that on uncontrolled oxidation a dark brown stable substance is produced. Perhaps the chlorogenic acid in the extract had been oxidized to compounds similar to those described by Steele. The passage of air through the hemlock needle extract did not seem to affect the yield of the other four phenolic acids from the potassium hydroxide fusion.

The above results reveal that the hemlock needle extract contained large amounts of phenolic compounds or compounds that have a phenolic constituent. Chlorogenic acid alone accounted for almost 20 per cent of the organic matter in these solutions. Since these compounds were not found in the hemlock leachate, it is apparent that there were large differences between the freshly prepared hemlock needle extract and the hemlock leachate. The passage of air through the hemlock needle extract altered its properties considerably. The resulting solution more closely resembled the hemlock leachate than did the freshly prepared material. This does not mean that passing air through a hemlock extract produces materials such as those found in a hemlock leachate. Nevertheless, the changes in the aqueous extracts of ground needles may, in some degree, parallel those occurring as needles age and die on the tree, when enzymatic reactions run wild and produce hydrolysis of glycosides. Also, periodic wetting and drying under the aerobic conditions of the O layer may well result in the production of brown polymeric substances. The latter are characteristic products of the condensation of quinones resulting from enzymatic oxidation of phenols (16). Such changes also might occur in needles detached by icing in winter or in violent storms. The author has observed many green hemlock needles on trays under hemlock canopy after a heavy rain.

Since none of the eleven phenolic materials was found in the hemlock needle extract or the canopy drip solution, with the methods of concentration used, there is reason to doubt any conclusion reached on the podzolization process when leaf extracts alone are used.

CONCLUSIONS

Preliminary work showed that appreciable amounts of organic matter passed through the two A horizons studied with each rainfall of sufficient magnitude to produce a leachate. These leachates also carried small amounts of iron. The relatively constant ratio of iron to organic matter in both leachates suggested that the movement of iron is closely related to the movement of organic matter through these horizons. It is evident that the iron-leaching process of podzolization is still going on in these soils. Much more organic matter moved through the A horizon under hemlock than through the A horizon under beech. This would indicate, since the ratio of iron to organic matter was almost the same for the two types of solutions, that podzolization was more active under hemlock canopy than under beech canopy.

An unsuccessful attempt was made to isolate a fraction of the organic matter from the A horizon leachates having properties similar to those recorded by DeLong and Schnitzer (21) for the alcohol-insoluble fraction they obtained from extracts and leachates of poplar leaves. The lack of similarity in characteristics of extracts and leachates from poplar leaves and of A horizon leachates is not unexpected when the difference in the nature of the materials leached is taken into account.

In electrophoretic studies on the hemlock leachate, it was found that the solution contained four detectable components. Two of these components contained aromatic materials. Four electrophoretic components were also found in an aqueous extract of a sample of the B horizon of the podzol under study which with the methods of detection used were very similar to those of the hemlock leachate. These findings suggest that organic materials passing through the eluvial horizon have been accumulated in the illuvial horizon.

A fractionation procedure was developed which separated both the hemlock leachate and B horizon extract into four fractions. One fraction in each of the two solutions appeared to be composed entirely of one of the electrophoretic components detected with paper electrophoresis. This material had a high electrophoretic mobility and held much more iron in solution than did other fractions of organic matter. The results would indicate that this material was relatively rich in negative charges. Perhaps for this reason it may be the most active fraction of the organic matter in the podzolization process.

The fractions of the hemlock leachate and B horizon extract were partially characterized by analysis. The analyses showed that there were only small differences in nitrogen content between the three major fractions of each solution or between corresponding fractions of the two solutions. Qualitative amino acid analyses showed that the composition of the amino acids was uniform throughout these fractions of each solution and was the same for both the hemlock leachate and B horizon extract. The carbohydrate content was more variable in the fractions although it never attained a high percentage in any fraction. The carbohydrate content of the hemlock leachate was somewhat higher than that of the B horizon extract. It would seem from these results that neither nitrogenous or carbohydrate materials accounted for a large percentage of the organic matter in these solutions.

Several materials giving positive reactions for the presence of phenolic groups were detected in the hydrolyzates of the fractions of the hemlock leachate and B horizon extract solution by means of paper partition chromatography. These phenolic materials were not uniformly distributed in all of the fractions of the hemlock leachate. The fractions of the B horizon extract showed less variation in phenolic materials. Nearly all of these phenolic materials were common to both the hemlock leachate and B horizon extract. The fraction of each solution thought to be the most active in the podzolization process produced almost identical chromatograms. None of these phenolic materials was analyzed either quantitatively or qualitatively. However, it was anticipated, since the total carbohydrate and nitrogenous content of hemlock leachate and B horizon extract was low, (7.4 and 7.0 per cent, respectively) that aromatic material might constitute a large percentage of the total organic matter in these solutions.

The organic matter in the hemlock leachate was fused with potassium hydroxide. Up to 35 per cent of the organic matter of the leachate was extracted into ether after the fusion. Four phenolic acids were identified which accounted for 6.2 per cent of the original organic matter. It is not possible to tell from what compound these phenolic acids were derived. It can only be stated that they came from aromatic substances. It is most likely that the leachates contain a much larger percentage of aromatic materials than this result would indicate.

It was shown by paper partition chromatography that after fusion the B horizon extract also yielded the same four phenolic acids in approximately the same percentage as did the hemlock leachate. This was additional evidence that the organic matter extracted from the B horizon resembled that in the hemlock leachate. Earlier results including the carbohydrate content, the nitrogen content, the amino acid composition, the phenolic materials found in acid hydrolyzates, the results of electrophoretic studies and of fractionation of the organic matter pointed to the similarity of the B horizon extract and the hemlock leachate. These results suggest that the organic matter, after precipitation from the leachates passing through the A horizon of this soil, was not altered greatly.

Hemlock needle extracts were examined. It was found that these extracts were quite acidic. Eleven phenolic compounds were detected in these extracts by means of paper partition chromatography. One of these, identified as chlorogenic acid, accounted for approximately 20 per cent of the organic matter in the solution. Since none of these materials was detected in the hemlock leachate, it is evident that the hemlock needle extract differed greatly from the hemlock leachate.

CLAIMS OF CONTRIBUTIONS TO KNOWLEDGE

1. Experimental evidence has been obtained in support of the view that the podzolization process is more active under a conifer (hemlock) than under a deciduous (beech) canopy.

2. A method has been developed for the separation, from very dilute aqueous leachates, of nearly all of the organic matter contained in them.

3. A method has been developed for the extraction of organic matter from a podzol soil by means of a cation exchange resin.

4. A chemical fractionation procedure has been developed which has served to isolate, from both a hemlock leachate and a B horizon extract, a substance which moved as a unit in electrophoresis, and had properties indicating that it was potentially active in podzolization.

5. Amino acids and phenolic materials were found in acid hydrolyzates of hemlock leachates and B horizon extracts.

6. Phenolic acids produced by alkali fusion of hemlock leachates and B horizon extracts were identified.

7. Evidence was obtained that chlorogenic acid, as such, is not an important factor in podzolization of soil under hemlock canopy.

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