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Characterization of metal-organic complexes in aspen and birch forest soils in interior Alaska

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CHARACTERIZATION OF METAL-ORGANIC COMPLEXES IN ASPEN AND BIRCH FOREST SOILS IN INTERIOR ALASKA

A

THESIS

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DOCTOR OF PHILOSOPHY

By
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CHARACTERIZATION OF METAL-ORGANIC COMPLEXES IN ASPEN AND BIRCH FOREST SOILS IN INTERIOR ALASKA

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ABSTRACT

Complexation of iron, copper, and manganese by organic substances contained in aqueous soil extracts obtained from the mineral B horizon of contiguous aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) forests was characterized by gel-permeation chromatography (GPC), thin-layer chromatography (TLC), ion-exchange chromatography, atomic absorption spectrometry, and infrared spectroscopy. Fractionation of these extracts on Sephadex G-25 (medium) yielded 6 fractions for birch extracts and 5 fractions for aspen extracts. This result indicated that the chemical composition of the birch extracts differed from the chemical composition of the aspen extracts. The largest quantity of metals was found in fractions that represented substances with apparent molecular weights less than 5000 daltons. Metal distribution patterns indicated different metal-organic associations within fractions of a forest-type extract as well as between fractions of the two forest-type extracts.

Fractionation of each GPC fraction on thin-layer plates yielded a total of 27 TLC bands for the birch GPC fractions and 19 TLC bands for the aspen GPC fractions. This result indicated that fewer components were separated in the aspen GPC fractions than in the

birch GPC fractions. Solutions derived from the TLC bands, when passed through a Chelex 100 column, provided qualitative information regarding the strength of copper, iron, and manganese complexation by organic substances in those solutions. Manganese was not strongly bound by organic substances derived from birch soil or aspen soil. Copper and iron were usually strongly complexed by organic materials regardless of the source. Significant differences in copper and iron complexation were observed within a forest-type soil extract and between forest-type soil extracts. TLC bands that exhibited little evidence of undissociated carboxylic acid character, as revealed by infrared spectroscopy, contained most of the iron and suggested that carboxylate anion was the principal complexing moiety for iron. Copper generally appeared to be concentrated in TLC bands that fluoresced which was in contrast to those containing iron. This result suggested separations of copper complexes from iron complexes.

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ACKNOWLEDGEMENTS

The writing of a thesis requires the patience of Job. This patience is not required of the author but everyone associated with the author in any way. Employers, supervisors, friends, and relatives must be willing to tolerate the author's cranky behavior, self-centered behavior, and other idiosyncrasies too numerous to mention. The rantings and ravings of the author, seemingly a certified lunatic, must at times be most unnerving.

I would like to thank Dr. Keith Van Cleve, my research advisor, for having the patience of Job. It was his encouragement and faith in my abilities that provided the impetus for initiation and completion of this study. He afforded me opportunities, when I was a technician, usually not available to technicians. While enrolled in the present graduate program, I was given opportunities for studies abroad unusual for most graduate students.

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I have enjoyed my association with everyone in the Forest Soils Lab. They never let you take yourself too

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A very special thank you goes to my parents. They inspired me to appreciate and to desire knowledge. They gave me inner strengths necessary to set goals and to achieve those goals.

General Introduction

Soils are characterized by a given sequence of layers called horizons. These layers include the organic (O), the eluvial (A or E), the illuvial (B), and the mineral (C) horizons. The O horizon may consist of slightly decomposed, moderately decomposed, and highly decomposed plant and animal residues. The eluvial horizon (A) is the topmost mineral horizon containing a strong admixture of low to high molecular weight decomposition products or humified organic matter. A zone of maximum accumulation of clays and hydrous oxides containing less organic matter than the A horizon constitutes the B horizon. The C horizon contains unconsolidated material underlying the A and B horizons, contains very little organic matter, and is outside the zones of major biological activity. A vertical exposure of these horizons is defined as a soil profile.

Nutrients and water soluble organic substances are translocated as water percolates through these horizons. Some of the metallic nutrients may be in the form of metal-organic compounds known as complexes or chelates.

The sharing of two electrons between two atoms can result in a single bond to form a compound. Any atom, ion, or molecule that donates a pair of electrons to a

central atom or ion is called a ligand and forms a co-ordinate bond. The resulting compound is known as a complex. A metal-organic chelate is a complex where an organic ligand bonds with a central metal ion to form a ring structure.

Copper and iron are strongly bound by humic substances. Therefore it is probable that metal-organic chelates play an important role in processes which form soil (Stevenson, 1985). These chelates may also affect the availability and transport of micronutrients, such as iron, necessary for healthy plant growth (Lindsay, 1979 and Stevenson, 1985). Formation of insoluble chelates such as calcium oxalate provides an immobile nutrient pool preventing leaching of macronutrients like calcium from soil (Graustein et al., 1977). A wide variety of methods have been used to the study the complexation of metals by humic substances derived from soils and aqueous environments.

Several chromatographic methods that minimize alteration of original substances have been employed to examine chelates in recent years (Mantoura et al., 1978; Steinberg, 1980; Hanson, 1981; Mills et al., 1981; Candler and Van Cleve, 1982; Candler, 1985a,b; Donat et al., 1985; Mills et al., 1987). These methods include gel-permeation chromatography, C^{18} reverse phase liquid chromatography,

and thin-layer chromatography. Most of these methods have been applied to aqueous systems. Regardless of the origin of the materials under consideration this author is convinced that no one chromatographic method is adequate to provide chromatographically pure components contained in what are obviously very complex mixtures. A combination of two or more chromatographic methods is probably required to elucidate clearly the ligand structures and binding mechanisms of these chelates.

Several years ago a study was initiated in this laboratory with the intent to explain the different turnover rates of metals observed in aspen (*Populus tremuloides*) forest soils, Ca > Mg > Zn > Mn > Fe, as compared with paper birch (*Betula papyrifera*) forest soils, Zn > Mn > Mg > Ca > Fe (Van Cleve and Noonan, 1975). The guiding operational hypothesis for this study was that forest type specific suites of water soluble organic compounds capable of chelating metals may be, in part, responsible for the observed differences in rates of element cycling. Results of the initial study supported the hypothesis (Candler and Van Cleve, 1982). The data presented in the subsequent sections of this thesis strongly suggest different metal-organic complexes exist among components within a forest-type soil extract.

Chapter 1

Gel Permeation Chromatography and Atomic Absorption Spectrometry

Introduction

Metallo-organic complexes derived from aquatic or terrestrial sources are comprised of a myriad of organic ligands. Many studies have employed gel-permeation(GPC) or reverse-phase liquid chromatography to delineate the number and types of complexes in solution (Khan, 1970; Mantoura and Riley, 1975; Sposito et al., 1976; Kaurichev et al., 1977; Steinberg, 1980; Arzhanova et al., 1981; Mills et al., 1982; Candler and Van Cleve, 1982; Gregson and Alloway, 1984; Donat et al., 1985; Schierl et al., 1986). Studies employing GPC have demonstrated that various metallo-organic complexes can be separated into chromatographic fractions according to apparent molecular weights ranging from less than 500 daltons to more than 10,000 daltons. The GPC chromatograms in these studies suggested that many fractions were composed of several unresolved components. This condition was demonstrated by shoulders on narrow peaks, very broad peaks, or very shallow troughs between peaks.

Mantoura and Riley (1975) noted a considerable difference in complexing ability of fulvic acids derived from fresh water and peat. In fact, in reviewing the

literature, one finds that fractionation patterns and metal-organic associations vary according to the source of material chromatographed regardless of aquatic or terrestrial origin. Vegetation type may affect the number and type of components found in extracts of soils. Van Cleve and Noonan (1975) observed marked differences in rates of movement of Ca, Mg, Zn, Mn, and Fe in birch (*Betula papyrifera*) and quaking aspen (*Populus tremuloides*). Candler and Van Cleve (1982) hypothesized that these rate differences were in part attributable to vegetation-type-specific suites of mobile, water-soluble chelates, and the results of their study supported this hypothesis. The objectives of the present study were to ascertain consistencies in GPC fractionation within, as well as between, the two forest-type extracts, and to evaluate consistency in distinct metal-organic associations within and between these extracts. Furthermore, GPC was employed on a preparative scale to obtain sufficient material in each fraction for future thin-layer chromatographic separations.

The GPC fractionation patterns of aqueous extracts of the mineral B horizon from the aspen and birch forests were consistent over a two-year period and agreed with results of Candler and Van Cleve (1982). These patterns consistently indicated dissimilar components

that make up the extracts from different forest types. Metal contents (Ca, Mg, Cu, Fe, Mn) found in each GPC fraction exhibited similar patterns within a forest type over the two-year study period. A number of differences were observed between the two forest types.

Methods and Materials

Sample Collection and Processing

Samples of the mineral B horizon were collected from contiguous aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) forests on a biweekly basis, begining in mid-July and ending the last week in September. An attempt was made to disperse sampling sites without bias. The field fresh samples were sieved, extracted and concentrated according to the method described by Candler and Van Cleve (1982). All biweekly extracts were combined within vegetation types and concentrated further yielding one pooled concentrate for each vegetation type. Pooled concentrates were necessary to ensure adequate quantities of dissolved material for all subsequent analyses. These pooled concentrates were considered to be representative of the water soluble materials to be found in soils since 5-6 individual samples were obtained from each forest type. The B horizon was chosen because water soluble materials in this horizon should constitute the most degraded and mobile compounds.

Gel Permeation Chromatography(GPC)

Fractionation of the concentrated extracts was done on a 120 X 2.5 cm column of Sephadex G-25(medium). The void volume (V_0 = 250 ml) was determined by blue dextran. Aliquots (4-6 ml, representing about 200-300 mg of dissolved material) of concentrate were placed on the column by means of a syringe fitted to a two-way valve at the head of the column. Dissolved material was eluted with distilled, doubly deionized, degassed water (DDDW) (pH = 6.9) at a flow rate of 60 ml/min. The resulting eluant was collected as 5 ml aliquots after the void volume and percent transmittance of each aliquot was determined at 362 nm in a 1 cm quartz cell using a Beckman model DBG spectrophotometer. Elution curves were prepared from these data for each chromatographic run. Several runs were required for each concentrate.

Appropriate aliquots comprising each Sephadex fraction from each chromatographic run were combined within a vegetation type, reduced in volume on a Buchi model R rotoevaporator, filtered through a 0.22 μm Falcon sterile filter unit, and stored at 4°C for future analysis. It was necessary to pool the fractions from each chromatographic run to ensure sufficient quantities of material for all subsequent analyses. A small portion from each fraction was removed with a syringe and freeze-dried for the determination of total

dissolved solids. About 5 ml was removed from each fraction, prior to volume reduction, for metal analysis by atomic absorption spectrometry.

Atomic Absorption Spectrometry

Copper, iron, and manganese were determined on a Perkin-Elmer model 5000 atomic absorption spectrometer equipped with a graphite furnace, model HGA 500, and autosamplers models AS-50 and AS-40. Calcium and magnesium were determined, in the presence of La_2O_3 as an interference suppressant, by the flame method.

Results and Discussion

Gel Permeation Chromatography

Soil extracts are complex mixtures of a wide variety of substances, so fractionation on Sephadex should reveal qualitative differences, if any, between the two forest-type extracts. It should also provide simplified mixtures for further separation using thin-layer chromatography. Representative elution curves for each year are shown in Figures 1-1 and 1-2. The appearance of the column, during fractionation of the aspen and birch samples, was very similar to that observed by Candler and Van Cleve (1982), and Candler (1985a). Six fractions (B1-B6) were observed for the birch extract whereas only 5 (A1-A5) were observed for the aspen extract. In themselves, the elution curves for the birch extracts did not suggest 6 fractions but there was a distinct

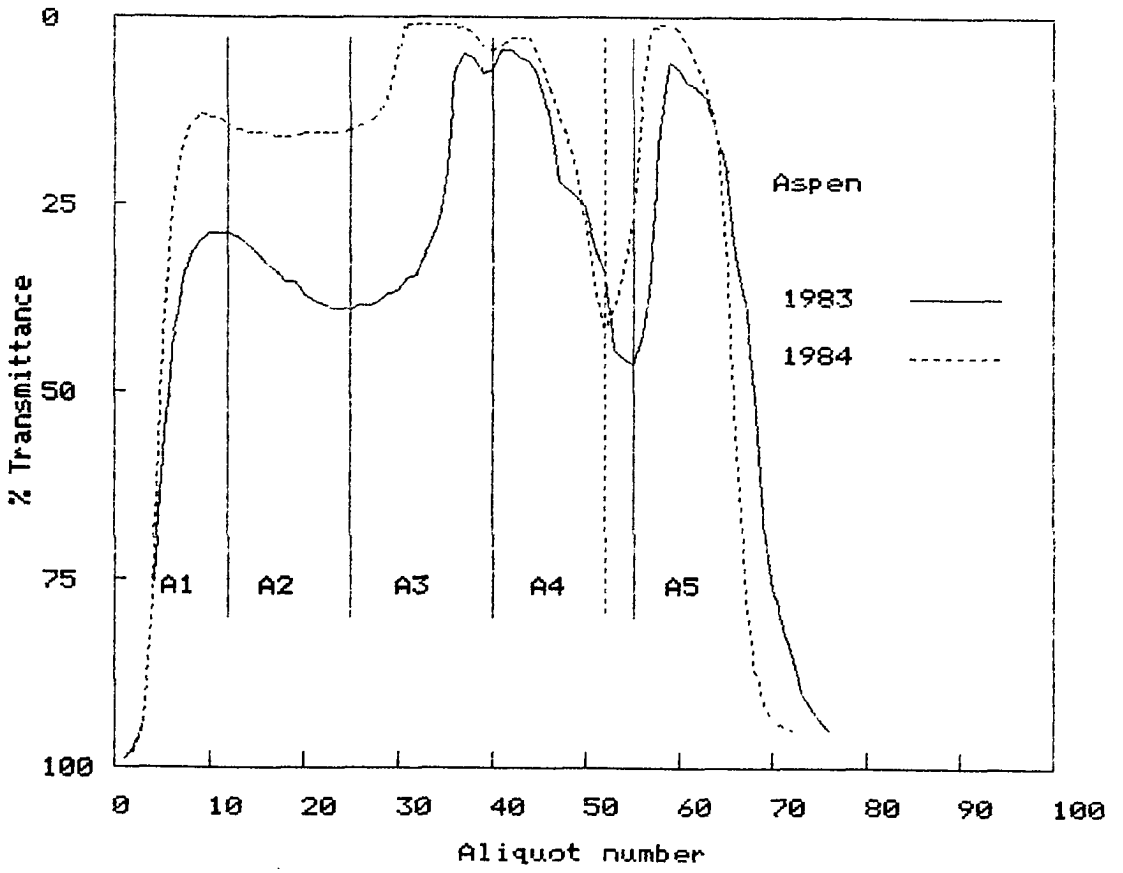

Figure 1-1. Representative elution curves for gel-permeation fractionation of 1983 and 1984 aspen soil extracts.

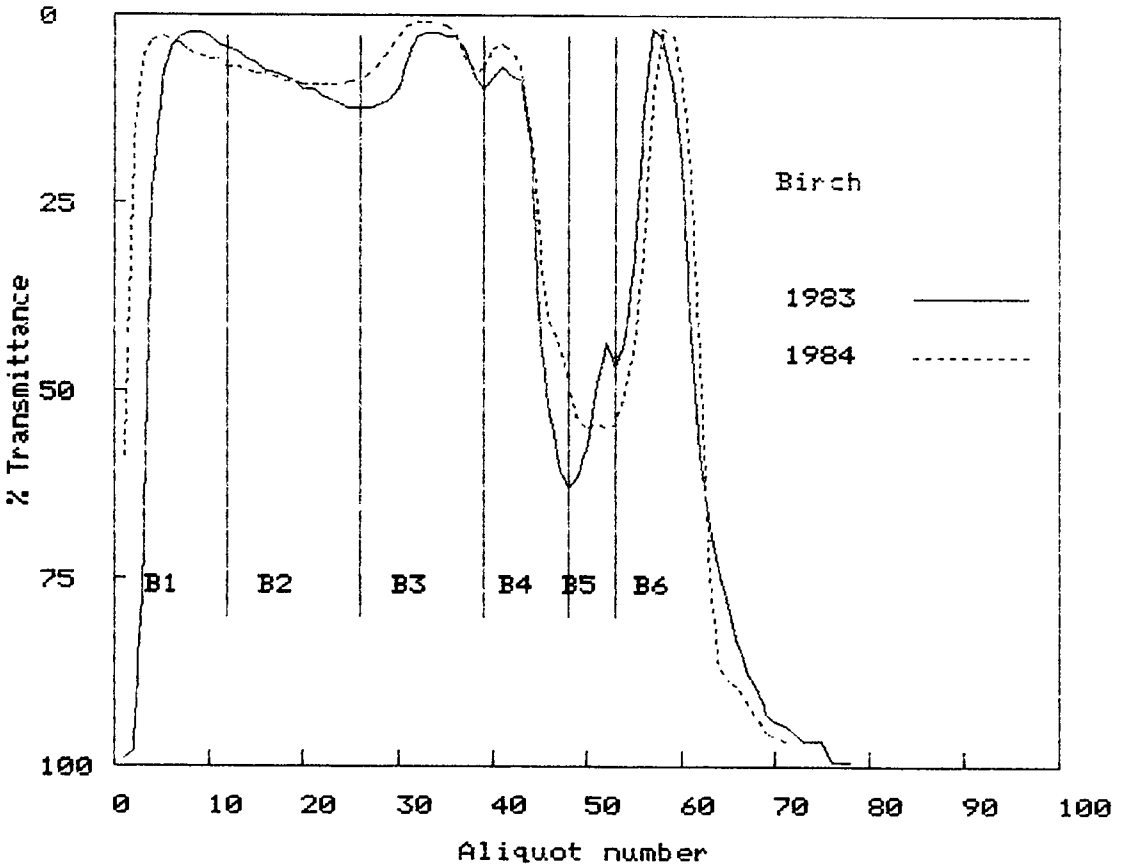

Figure 1-2. Representative elution curves for gel-permeation fractionation of 1983 and 1984 birch soil extracts.

color band corresponding to the trough between aliquot numbers 49 and 55 (fraction B5). This phenomenon was not observed during any of the aspen runs.

Two characteristic differences in the elution curves readily distinguish aspen extracts from birch extracts. First, percent transmittance values for the first two fractions of aspen were considerably higher than birch. Percent transmittance may be converted to absorbance units by the equation $A = \log_{10}(1/T)$, where T = percent transmittance/100. Absorbance is proportional to the absorptivity and concentration of the solute. Absorptivity is dependent upon the nature of the absorbing material or the chromophores present in the substance and the wavelength at which percent transmittance measurements were obtained. The elution curves were prepared from the percent transmittance measurements at a constant wavelength. Therefore, absorbance values obtained from the conversion of percent transmittance are directly proportional to the types of chromophores present in the solute and concentration of the solute. The ratio of maximum absorbance of GPC fraction 3 to GPC fraction 1 within a forest type may be used to compare differences in solute concentrations or the chromophores present in GPC fraction 1 between forest types. This ratio was 2.3 or greater for aspen and 1.4 or less for birch. The difference in these ratios

indicated that, if these were the same substances, they were present in lower concentrations in aspen extracts or they were similar in apparent molecular size but with sufficiently different chromophores to imply the existence of dissimilar molecular structures in the extracts from the two forest types. Secondly, more unresolved components were apparent in aspen extracts than birch (figures 1-1 and 1-2). Fraction four (aspen) contained at least 3 unresolved components whereas birch contained two. The last aspen fraction contained at least two unresolved components and there appeared to be no unresolved components in the last fraction for birch. Many more unresolved components may be present in these fractions but they are not apparent as evidenced by shoulders on the main peaks. Table 1-1 summarizes the elution characteristics of birch and aspen extracts for two years (1983 and 1984).

Several factors affect GPC separations. Hine and Bursill (1984) demonstrated the effects of salts and eluants on fractionation of humic acid. GPC separations were greatly enhanced in the presence of salts regardless of the eluant used. Buffered eluting solvents effected better separations. The poorest separations were obtained when water was used as the eluting solvent in a salt-free environment. Ionic strength can affect separations by charge induced exclusion from the gel beads. Gel-solute

Table 1-1. Elution Characteristics of Birch and Aspen Soil Extracts Average K_d values for all chromatographic runs.

Birch 1983				Aspen 1983			
Fraction	**K_d**	**SD**	**%TDS**	**Fraction**	**K_d**	**SD**	**%TDS**
1	0.00	0.02	11.6	1	0.00	0.02	21.4
2	0.17	0.02	14.9	2	0.18	0.02	11.0
3	0.40	0.02	23.7	3	0.41	0.02	17.5
4	0.62	0.02	19.6	4	0.61	0.02	27.9
5	0.76	0.01	2.8	5	0.84	0.03	12.2
6	0.82	0.02	18.7			**Total**	90.0
		Total	91.3				
Birch 1984				**Aspen 1984**			
1	0.00	0.00	15.1	1	0.00	0.00	8.3
2	0.19	0.02	11.7	2	0.20	0.04	4.2
3	0.39	0.04	23.4	3	0.36	0.03	26.0
4	0.60	0.01	20.7	4	0.63	0.02	27.9
5	0.78	0.03	5.9	5	0.84	0.02	22.1
6	0.86	0.05	14.9			**Total**	88.5
		Total	91.7				

$K_d=(V_e-V_0)/V_i$, where Vi=calculated internal volume if the gel bed, V_e= elution volume, and V_0=void volume.
SD=standard deviation; %TDS=% of total dissolved solids on column.

interactions such as adsorption can also affect separations. This influence leads to ambiguous interpretation of molecular weights from GPC chromatograms (Gjessing, 1976; Saito and Hyano, 1979; Miles and Brezonik, 1983). On this basis, Hine and Bursill (1984) considered fractionation on Sephadex gels to be of limited value in the comparison of aqueous humic substances.

The observed separations may not be due to molecular size alone since the effect of inorganic salts in each of the extracts of this study could not be discounted. Consequently, the separations did not lend themselves to molecular weight estimations. It has been suggested that, to minimize sample modification, fractionation should be carried out at ambient concentrations with distilled water (Aho, 1986; Gardner and Landrum, 1983). Sample pretreatment by acidification, for example, would most likely produce changes in the naturally occurring speciation of the metals to be studied (Steinberg, 1980). In fact, artifacts may be generated when acidic or basic eluants are used since many chelates may be quite sensitive to pH changes. Furthermore, extraction by a strong base can produce "chemical artifacts", a phenomenon first demonstrated by Wiesemuller (1967) and further corroborated by Khairy and Ziechmann (1981). The concentration procedure used in this study, which was a 600 to a 1000 fold concentration, resulted in a pH drop of

0.3-0.5 pH units and could conceivably have modified original components in the extracts. However, it was decided that these conditions were mild enough, compared with acidic or basic extractions, that any alteration of original material would be minimal.

Lehto et al. (1986) established that pure water as an eluant yielded the best separation for naturally occurring aquatic humic substances at ambient concentrations, a result which is in contrast to the findings of Hine and Bursill (1984). Distilled, deionized, degassed water was used in this study because of the sensitivity of chelates to drastic pH changes and to minimize artifact generation. Since it is unclear that separations on Sephadex are due to size alone, any references to molecular weights are considered to be apparent molecular weights (high, $K_d=0$; intermediate, $0<K_d<0.7$; and low, $K_d>0.7$). High molecular weight substances are considered to be 5000 daltons or larger. In all cases most of the material eluted fell in the intermediate to low molecular weight range, a little over fifty percent of these substances being in the intermediate molecular weight range.

The percentage distribution, expressed as percent of total dissolved solids (%TDS), for birch was very similar for the two years, as expected from the appearance of the elution curves. The aspen elution

curves were also similar in appearance for both years. Consequently, one would expect the percentage distributions to be nearly the same for the 1983 and 1984 samples. The apparent percent distribution variability, observed for fractions 1 to 3, and 5 for the two years, in aspen is unclear (Table 1-1).

The minimum number of components seemed to be consistent for both forest types for both years (see Figures 1-1 and 1-2). In all cases the minimum number of components comprising fractions 1 and 2 was indeterminant. The first fraction in all fractionated extracts was eluted at the void volume. This result suggested that this particular fraction was excluded from the gel beads, that it would possess a molecular weight greater than 5000 daltons, and that it probably was composed of several components.

These data indicated the presence of dissimilar molecular components between the two vegetation types although similar substances in differing quantites could not be dismissed. These were crude separations at best, and each band was probably composed of more than one component. The rationale for using GPC was to intially simplify the complex mixtures so that other separation techniques could be applied to define more clearly the metal-organic chelates found in soils.

Atomic Absorption Spectrometry

Figures (1-3) through (1-7) depict the distribution of each metal in each Sephadex fraction for both years and both forest types. The percentages are based on the total metal off the column since the mass of aspen Mg (1983, 1984) and Ca (1984) placed on the column are in question or not available. Percentage of Cu, Fe, and Mn off the column represent 80%-115% of these metals placed on the column. Visual inspection of figures 1-3 through 1-7 revealed that the distribution patterns of metal content observed for the respective extracts were very similar for both years within a forest type. Most of the metals were concentrated in the intermediate to low apparent molecular weight substances, a result similar to that of Steinberg (1980). Recently, Gardiner et al. (unpublished manuscript), using size exclusion chromatography to study organic and inorganic aluminum and iron speciation, found that Fe in particular was concentrated with the higher molecular weight substances derived from the A_h horizon. This result was explained on the basis that organic substances in this horizon were still undergoing more active degradation than substances in the B horizon and hence possessed more high-molecular-weight materials than might be expected in the B horizon.

In all cases for both years, maximum metal content was found in fractions 2, 3, or 4 (K_d=0.2, 0.4, and 0.6).

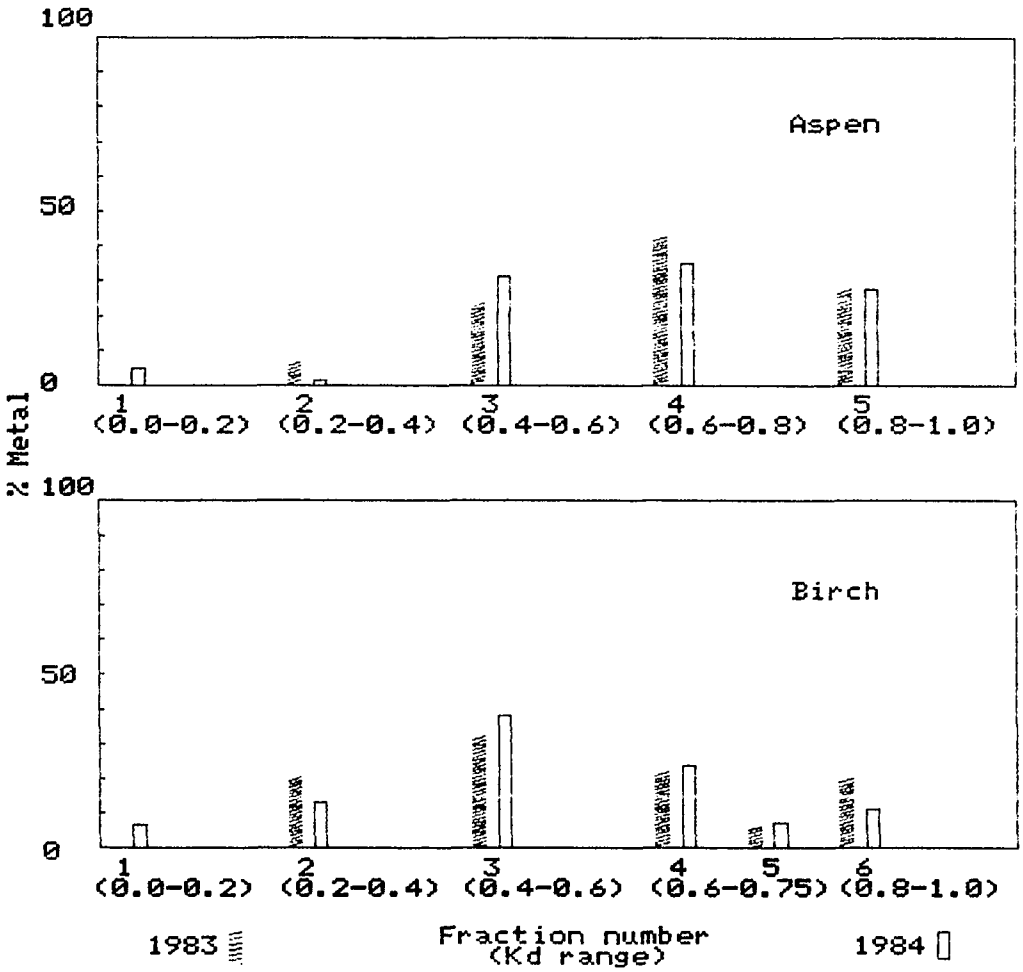

Figure 1-3. Distribution of calcium (Ca) among GPC fractions, expressed as percent Ca based on the total quantity eluted from the Sephadex G-25 (medium) column. Fraction 5 (Birch) K_d range is 0.75-0.8.

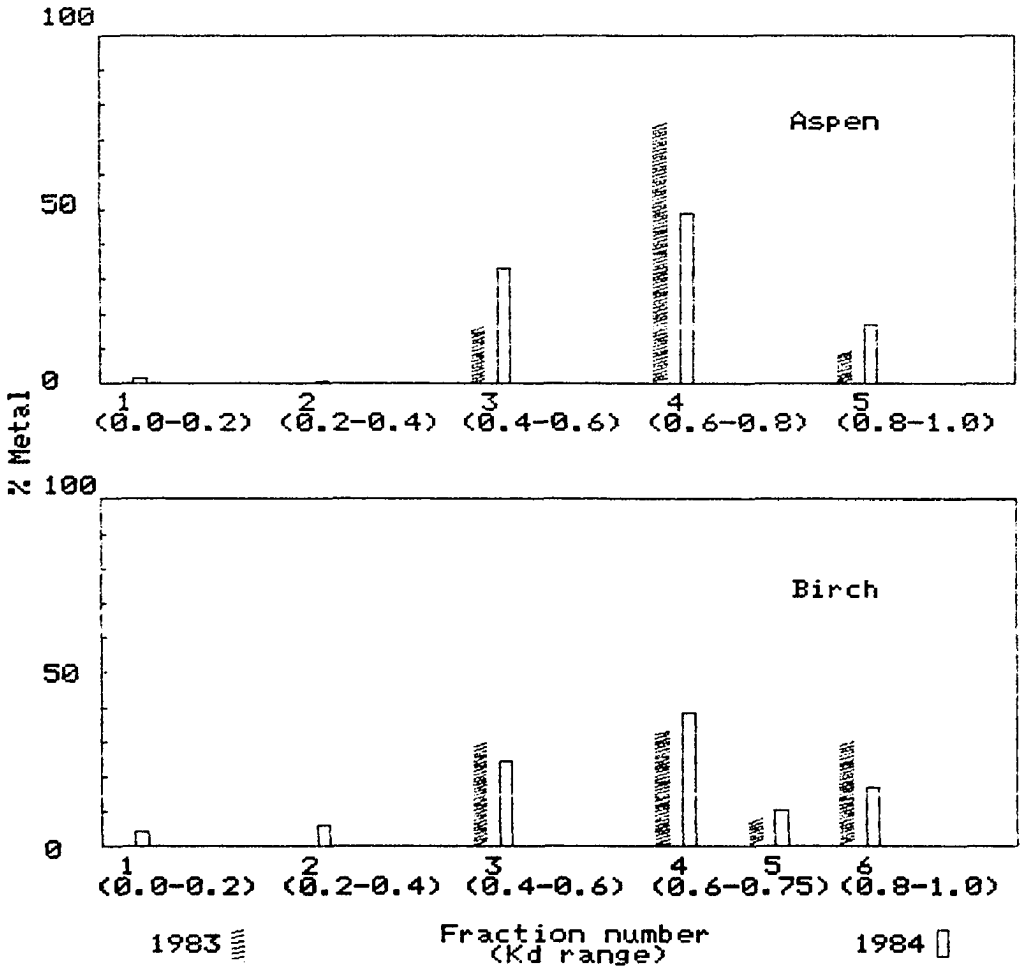

Figure 1-4. Distribution of magnesium (Mg) among GPC fractions, expressed as percent Mg based on the total quantity eluted from the Sephadex G-25 (medium) column. Fraction 5 (Birch) K_d range is 0.75-0.8.

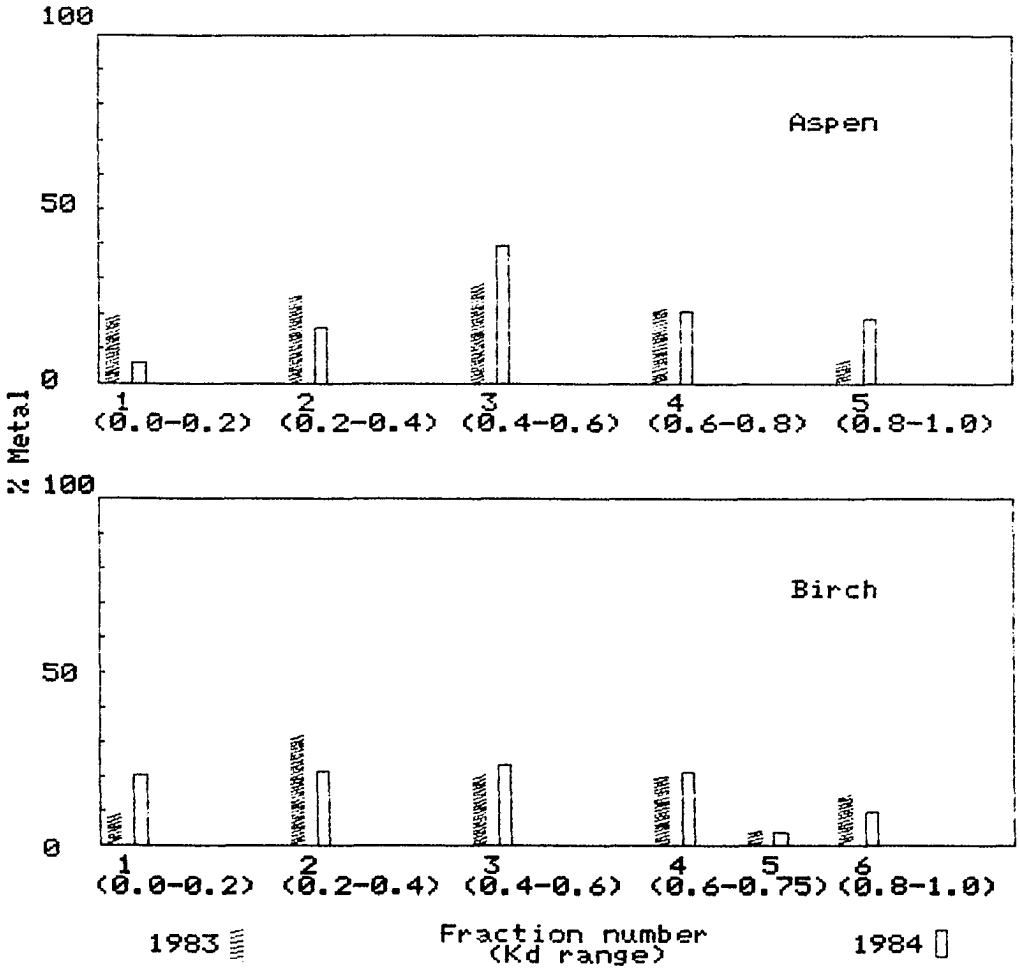

Figure 1-5. Distribution of copper (Cu) among GPC fractions, expressed as percent Cu based on the total quantity eluted from the Sephadex G-25 (medium) column. Fraction 5 (Birch) K_d range is 0.75-0.8.

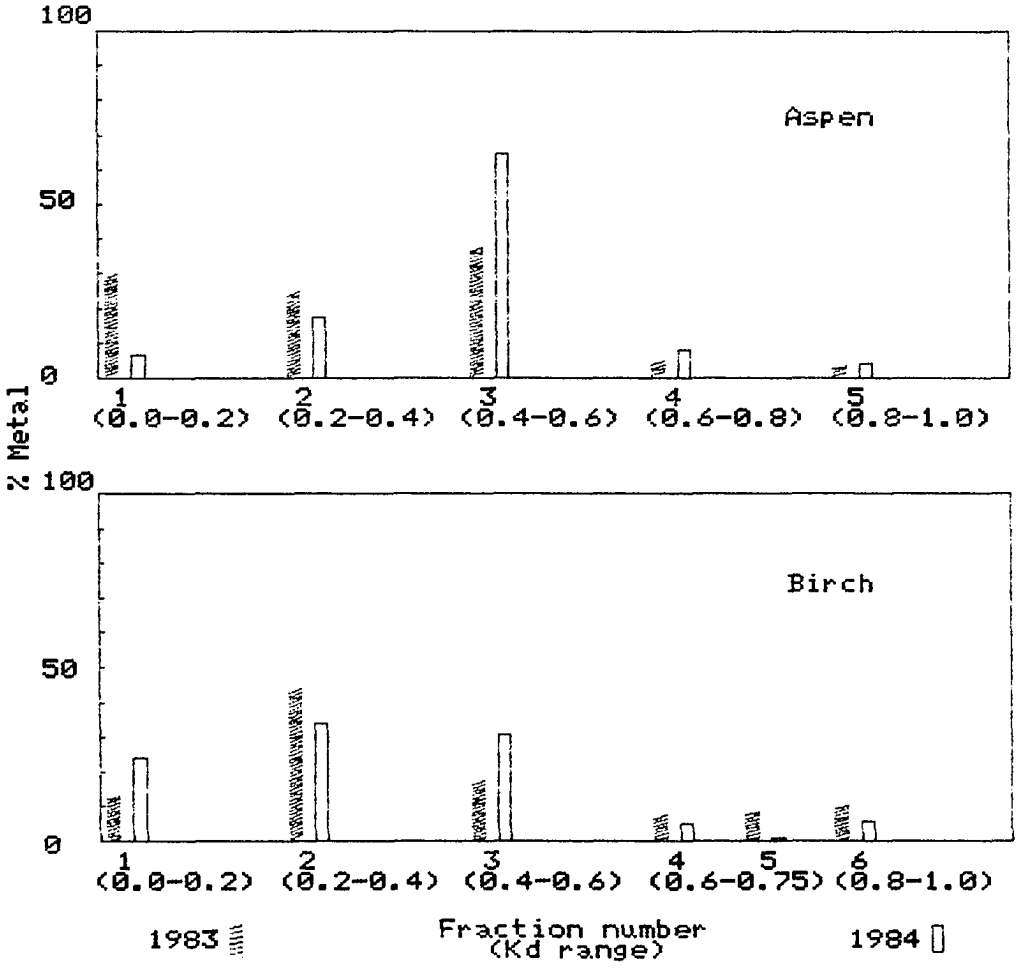

Figure 1-6. Distribution of iron (Fe) among GPC fractions, expressed as percent Fe based on the total quantity eluted from the Sephadex G-25 (medium) column. Fraction 5 (Birch) K_d range is 0.75-0.8.

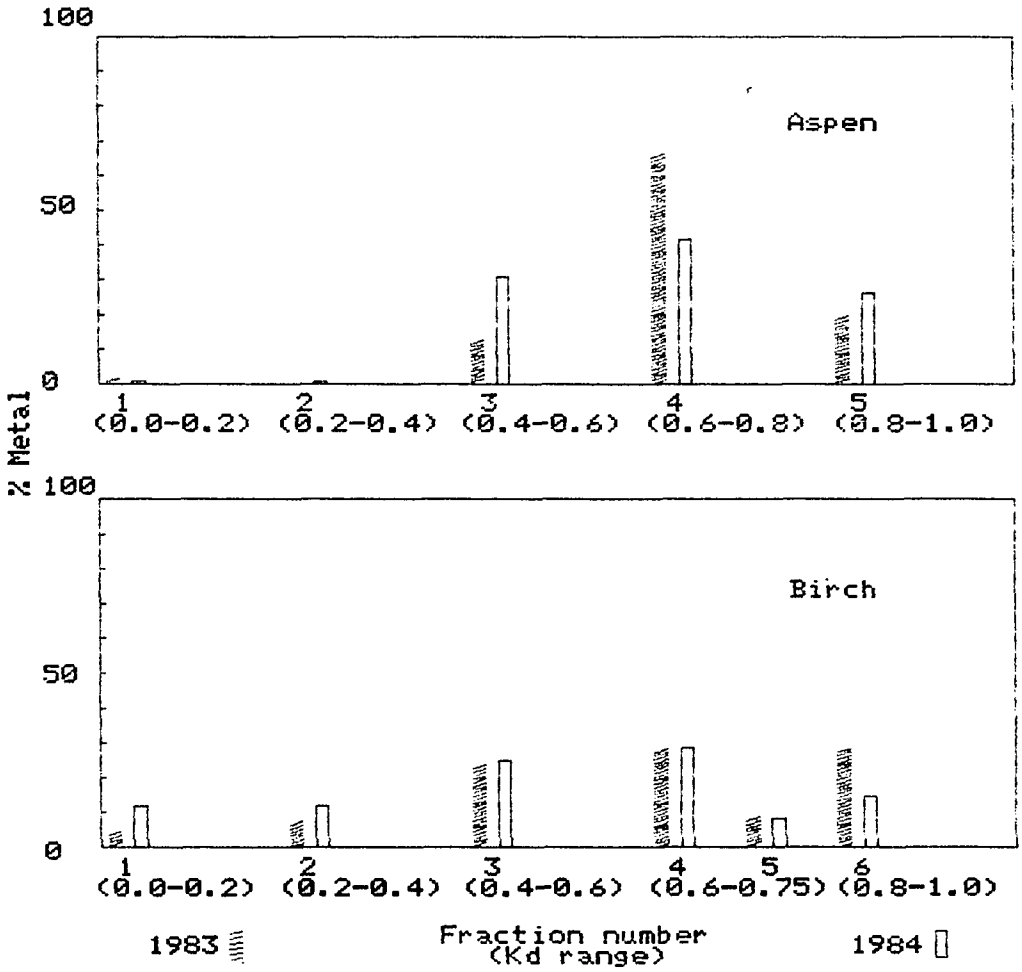

Figure 1-7. Distribution of manganese (Mn) among GPC fractions, expressed as percent Mn based on the total quantity eluted from the Sephadex G-25 (medium) column. Fraction 5 (Birch) K_d range is 0.75-0.8.

Therefore, most of the metals appeared to be associated with organic material of apparent intermediate molecular weight. Chromatographic behavior for Fe was apparently different for both years between the aspen and birch samples. Iron maxima occurred in the third fraction of aspen, whereas iron maxima were observed in the second fraction of the birch extract. This difference indicated that iron was primarily associated with substances of greater apparent molecular weight in the birch samples than in the aspen samples, and supported the idea that different substances chelated metals in aspen and birch soils.

Manganese in the birch samples (1983, 1984) exhibited similar chromatographic behavior to that of manganese in the aspen samples (1983, 1984). The maxima occurred in the fourth fraction. This result indicated that manganese was associated with lower molecular weight substances, and was partially separated from copper-organic and iron-organic materials. For both years, copper in the aspen samples exhibited maxima in the third fraction. In birch, copper behaved similarly to iron in the 1983 samples but was at a maximum in third fraction in the 1984 samples. This result indicated that production of organic substances possibly varied on a yearly basis.

Although Ca and Mg were not studied in detail, in all cases these metals were at maxima in either fractions

three or four. Calcium was at a maximum in fraction four of the aspen soil extract and in fraction three of the birch soil extract. Maximum magnesium content was observed in fraction four of the aspen soil extracts and the birch soil extracts. These relationships suggested possible dissimilar metal-organic associations between birch and aspen soil extracts, and among fractions within extracts.

Previous studies of aqueous soil extracts from aspen and birch forest soils, using spectroscopic techniques, have shown that there are some distinct dissimilarities among fractions within a forest-type extract as well as between the fractions of forest-type extracts (Candler and Van Cleve, 1982 and Candler, 1985a). Examples of infrared (IR) spectra obtained from those fractionated extracts are presented in Figures 1-8 and 1-9 (Candler, 1985a). These spectra further illustrate the differences in organic components contained in soil extracts originating from different sources. Principal dissimilarities, as evidenced by band contour and absorption intensities, were noted in the 1800-1600 cm^{-1} (carbonyl and COO^-) and 1200-1050 cm^{-1} (C-O and carbohydrate) regions, indicating that these structural units constitute the principal differences among fractions within a forest type and between forest types. Undissociated carboxylic acid functional groups generally give rise to broad

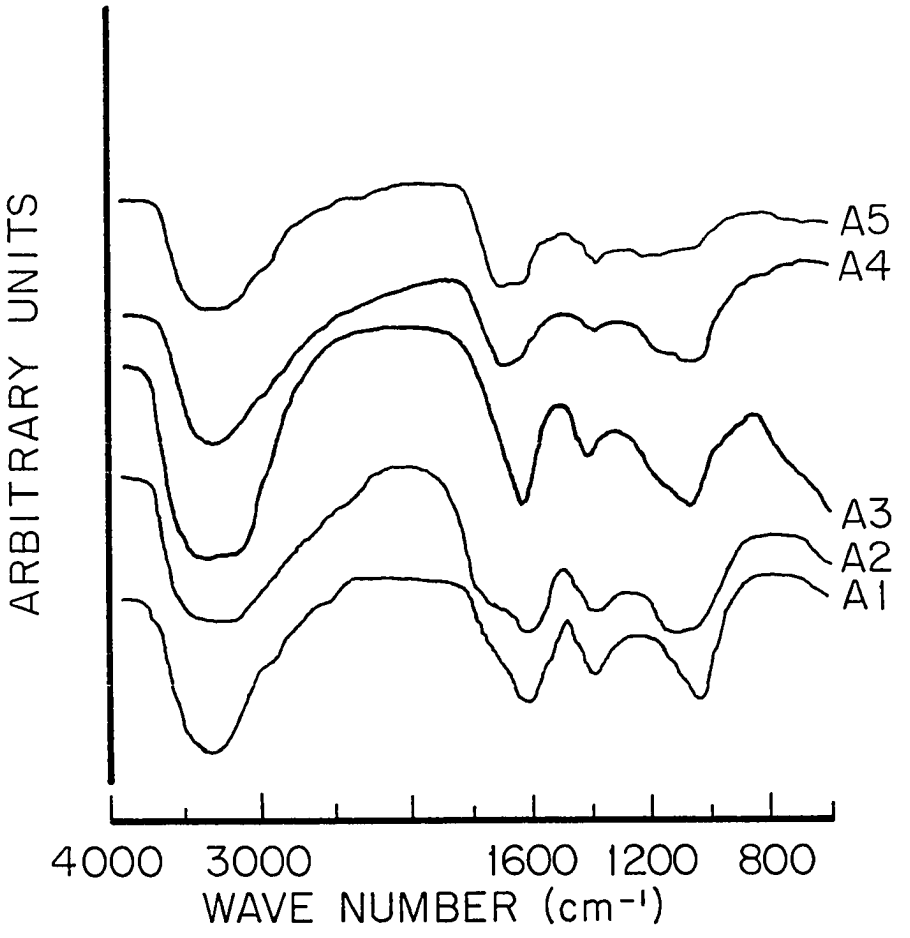

Figure 1-8. Infrared spectra of aspen gel-permeation chromatographic fractions A1, A2, A3, A4, and A5. All spectra were recorded as % Transmittance versus wave number (cm^{-1}) and placed in a single figure.

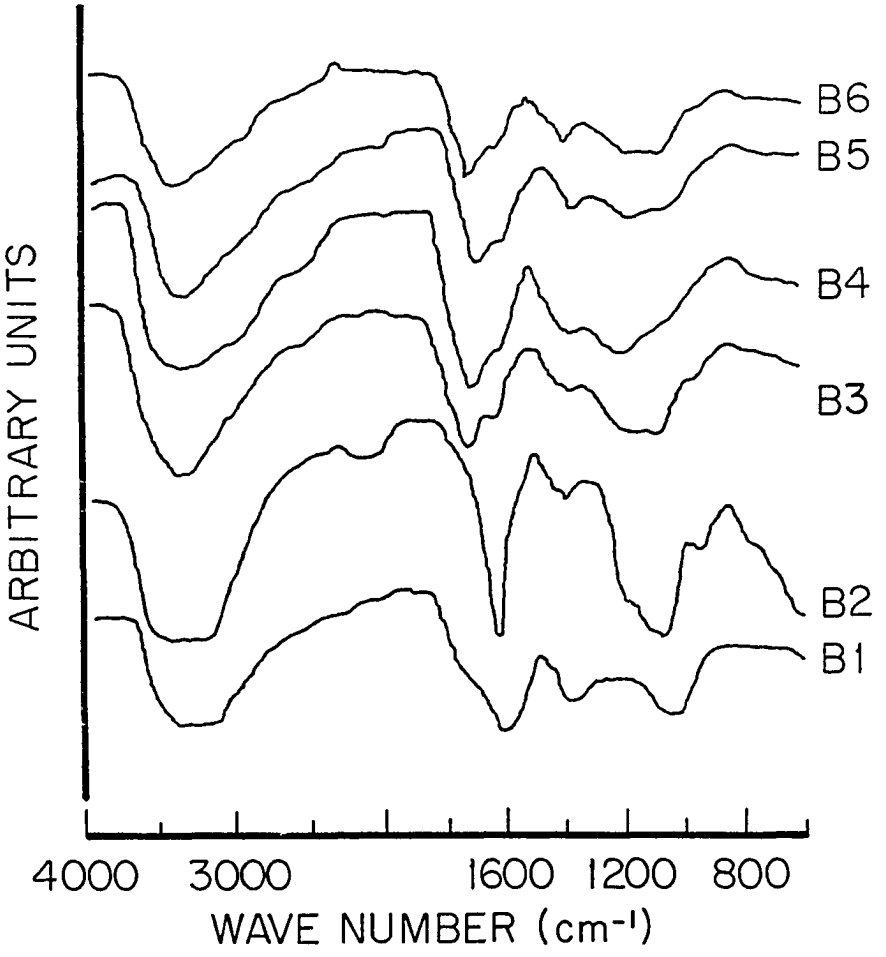

Figure 1-9. Infrared spectra of birch gel-permeation chromatographic fractions B1, B2, B3, B4, B5, and B6. All spectra were recorded as % Transmittance versus wave number (cm^{-1}) and placed in a single figure.

absorption from 3400-2500 cm^{-1} and strong absorption near 1720 cm^{-1} which is observed in all spectra except the third fraction of aspen and the second fraction of birch. Chelated metals such as copper and iron are thought to be bound by dissociated -COOH functional groups (Schnitzer and Khan, 1970) and these two spectra may indicate that most of the metal binding sites are occupied by metals. The lack of absorption near 1500 cm^{-1} suggests that aromatic or amide functional groups are not major contributors to overall structure of these substances (Nakanishi and Solomon, 1977; Bellamy, 1975). Percent transmittance may be converted to absorbance which is proportional to concentration. The ratio of absorbance attributed to undissociated -COOH near 1720 cm^{-1} to the maximum absorbance in the carbohydrate region (1150-1050 cm^{-1}) indicated that substances with decreased carbohydrate character showed more undissociated -COOH character. For example, birch fraction 2 exhibited very little undissociated -COOH character and strong carbohydrate character. This ratio was 0.2. Birch fraction 4 showed intense absorption near 1720 cm^{-1} and relatively weak absorption in the carbohydrate region. The ratio of these absorbances was 1.8 and indicated more undissociated -COOH character than carbohydrate character.

Conclusions

Conclusions drawn from the data presented here are:

1. Similar elution curves, based on the number of fractions and absorbance ratios of fraction 3 to fraction 1, were observed within each forest-type extract for 1983 and 1984. This result indicated that similar substances were produced on a yearly basis within a vegetation type.
2. Dissimilar elution curves between the two forest-type extracts over the two years of study indicated that there was a consistent difference in the organic chemical components comprising the soil extracts.
3. There was sufficient evidence in the metal distribution patterns to suggest distinctive metal-organic associations among fractions within a forest type extract as well as between fractions of the two forest type extracts.
4. Similar metal distributions within a forest type during the study period indicated fairly consistent metal-organic associations.

Chapter 2

Charaterization of possible metal-organic chelates in aqueous extracts from the B horizon of a contiguous aspen and birch forest in interior Alaska by thin-layer chromatography and infrared spectroscopy

Introduction

Gel-permeation chromatographic studies of water soluble metallo-organic complexes have demonstrated that each fraction was composed of several components (Khan, 1970; Kaurichev et al., 1977; Steinberg, 1980; Candler and Van Cleve, 1982; Gregson and Alloway, 1984). These results implied that individual GPC fractions were conducive to further separation or fractionation by other chromatographic methods. Reverse-phase liquid chromatography has been successfully used to separate copper-, cadmium-, iron-, manganese-, nickel-, and zinc-organic complexes from unfractionated samples of aqueous origin (Mills et al., 1981 and Donat et al., 1985, Mills et al.,1987). Aluminum-organic complexes from aqueous soil extracts have been determined using these methods (Schierl et al. 1986).

An inexpensive and relatively rapid method of chromatographic separation is thin-layer chromatography (TLC). Effective separation of metal-organic chelates via TLC has been established (Heizman and Ballschmiter, 1972; Niederschulte and Ballschmiter, 1972;

Ballschmiter, 1973; Oksala and Krause, 1976). The chelates separated were well characterized, and the structures were known. Therefore, the sorbent and mobile phase were easily selected. Metal-organic chelates derived from soil or aquatic sources are complex mixtures, poorly characterized, and their complete structures generally unknown. Moreover, possible surface reactions may occur with many TLC sorbents which can alter the original material. These facts dictate that the TLC sorbent should be one with essentially a non-reactive surface.

The purpose of this chapter is to describe the results of further separations of molecular components obtained by TLC. This work was conducted on the GPC fractions obtained from birch (*Betula papyrifera*) and quaking aspen (*Populus tremuloides*) soil extracts of the B horizon. A sorbent with a relatively inactive surface, Kieselguhr, which is porous silica derived from diatoms, was chosen to minimize possible alteration of the original substances applied to the plates. Characteristic migration patterns of these components on thin-layer plates and infrared spectra of the substances are presented for the respective TLC bands.

Methods and Materials

Sample Collection and Processing

Samples of mineral soil B horizon were collected from contiguous aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) forests. An attempt was made to disperse the sampling sites without bias. The samples were sieved, extracted and concentrated according to the method of Candler and Van Cleve (1982). Each concentrate was fractionated on Sephadex G-25 (medium) (see Chapter 1; Methods and Materials). Concentrated fractions obtained from gel-permeation chromatography (GPC) for the two years 1983 and 1984 were used for the analyses discussed below.

Thin-layer Chromatography(TLC)

TLC Procedure I(TLC I)

Two to three milliliters of each concentrated Sephadex fraction were applied to 20 X 20 X 0.05 cm pre-washed, commercially prepared, thin-layer plates as a streak 16 cm long and no more than 2 mm wide. The sorbent was Kieselguhr N. The concentrate was applied, under pressure at approximately 0.6 bar, using a Desaga Autoliner Model 75. Nearly 200-300 passes were required at 10 µl per pass to deposit 2-3 ml of sample. A setting of two drying cycles after each application of solution, under an additional stream of nitrogen gas, was necessary to prevent the applied material from spreading beyond the

2 mm width constraint. The best separations are obtained by TLC with a very narrow streak. The 2 mm width constraint provided reasonable separations. This process was repeated until all of the concentrates were applied to the TLC plates. The resulting number of plates varied according to the total volume of GPC-fraction concentrates and to the success of maintaining the streak constraints of 16 cm × 2 mm.

A mobile phase composed of n-butanol:water:2-propanol in a ratio of 5:5:3 was found to provide the best separation of components. At ambient temperature, the solvent was allowed to migrate to 12-13 cm (solvent front) from the origin. Following development, plates were dried under a hair dryer placed about 30 cm from the surface of the plates. Separation zones were observed under visible and UV light (366 nm) from which R_f values were determined.

TLC fractions were designated as: Forest Type (A = aspen and B = birch), GPC $\text{fraction}_{\text{Rf value}}$. For example, $B1_0$ represented the TLC band with R_f=0 of the first GPC fraction for a birch extract.

Elution of Samples from Plates

Each TLC band was scraped from the plates with a pyrex glass slide and placed in acid-washed polyethylene tubes. A small quantity of distilled-doubly-deionized water (DDDW) was added to each tube to form a slurry.

The slurry was filtered through acid-washed fiberglass packed in Pasteur pipettes, under nitrogen gas, until no color was observed on the sorbent. The effluent was collected in acid-washed polyethylene tubes under nitrogen gas. These filtrates were transferred to 25 ml volumetric flasks and brought to volume with DDDW. Filtrates exhibiting cloudiness due to Kieselguhr particles were centrifuged at 5000 rpm for five minutes, and the supernatant was decanted into 25 ml volumetric flasks and brought to volume with DDDW. Four replicate plates of the 1983 material were used for subsequent ion exchange chromatography studies. Only two replicate plates of the 1984 material were used for similar studies. The reason for the latter modification was to conserve as much material as possible for infrared analysis, a second TLC procedure(TLC II), and subsequent ion exchange studies.

TLC Procedure II(TLC II)

TLC bands from the remaining plates (1984 samples) with equivalent R_f values were scraped from the plates, and combined within a vegetation type. The material was then eluted from the sorbent using DDDW, as described above, and filtered through prewashed sterile filter units (0.22 μm). The resulting solutions were then freeze-dried. Bands of interest were $B2_{0.3}$, $A2_{0.3}$, $B3_{0.4}$, $A3_{0.4}$, $A4_{0.5}$, and $B4_{0.5}$ since they appeared to

contain the bulk of organic material based on their color density and band width.

A known quantity, between 6 and 15 mg of each sample, was dissolved in DDDW, applied as a streak (described previously) on prewashed commercially prepared TLC plates, and developed sequentially with 2-prOH:H_2O--7:3, 5:5, 1:2, 1:3, 1:5 to successively less distances from the origin. The plates were dried, as described earlier, after plate development by each carrier solvent. This procedure resulted in six distinct bands. Polarity of the mobile phase increased as water content increased. Four replicate plates were used, two for infrared studies, and two for for subsequent ion exchange studies. Bands were observed under UV (366 nm) and visible light. Two prewashed blank TLC plates were sequentially developed, as described earlier, for subsequent infrared spectroscopic studies.

Infrared Spectroscopy

Spectra were obtained for the TLC I fractionated material of 1984 GPC fractions A2, A3, A4, B2, B3, and B4 only. The substances comprising these fractions represented the bulk of material found in the extracts and were considered to contain the principal potential chelators. Approximately 1 mg of freeze-dried material was pulverized with 200 mg spectral of grade KBr, pressed into clear pellets, and the spectra recorded. All

spectra were recorded on a Perkin-Elmer Model 283B infrared spectrophotometer equipped with a data station. The spectral range was 4000-600 cm^{-1}.

Two solutions of each TLC II band were obtained as previously described and freeze dried on KBr. The resulting powder was pressed into a pellet and the spectra recorded. Blanks were obtained similarly and then subtracted from the sample spectra using the data station and software accompanying the infrared spectrophotometer.

Results and Discussion

TLC Plate Descriptions (TLC I)

Separations on Sephadex for the myriad of substances comprising aqueous soil extracts is crude at best. Thin-layer chromatography (TLC) was used to separate further components composing each Sephadex fraction for both years. These fractions were designated as B1, B2, B3, B4, B5, B6 (birch) and A1, A2, A3, A4, A5 (aspen). Since overlapping of TLC fractions was generally observed, each TLC fraction represents a zone of separation. A total of 27 zones of separation were observed for the TLC of B1-B6 and 19 for A1-A5. These results agree with results from a preliminary study on this site using similar methods (Candler, 1985b). The R_f values were determined as the distance from the leading edge of the streak applied to the plate, specified as the origin, to the leading edge of a noticeable band, divided by the

Table 2-1. Average R_f values for each TLC I band observed for each GPC fraction (1983 and 1984 samples) and the number of zones of separation.

	Forest Type			
	Aspen		Birch	
GPC Fraction	R_f	Number of Zones	R_f	Number of Zones
1	0.0, 0.2, 1.0	3	0.0, 0.1, 0.2, 0.9,1.0	5
2	0.0, 0.1, 0.2 0.9, 1.0	5	0.0, 0.1, 0.3 0.9, 1.0	5
3	0.0, 0.4, 0.9, 1.0	4	0.0, 0.1, 0.4, 0.8, 0.9, 1.0	6
4	0.5, 1.0	2	0.5, 1.0	2
5	0.0, 0.1, 0.3, 0.9, 1.0	5	0.0, 0.2, 0.8, 1.0	4
6	----------	-	0.1, 0.3, 0.6, 0.9, 1.0	5
	Total	19		27

R_f=(distance(cm) of band from origin)/(distance(cm) of solvent front from origin)

distance of the solvent front from the origin. Table (2-1) summarizes the TLC separations carried out in this study for both years (1983 and 1984).

The first four GPC fractions for both aspen and birch soil extracts displayed considerable overlap. Therefore, TLC bands with the same R_f values may be the same substances, especially in the case of those TLC bands of the first four GPC fractions. For example, $B1_0=B2_0=B3_0$, $B1_1=B2_1=B3_1=B4_1$, $A1_0=A2_0=A3_0$, etc. The presence of dissimilar substances was indicated where R_f values clearly differed within, as well as between, vegetation types. Therefore, it was unlikely that any TLC bands of GPC fraction A5, with R_f=0, 0.1, 0.3, or 0.9, contained the same substances found in other TLC bands with equivalent R_f values observed in GPC fractions A1-A3. The reason for this is that only two bands, R_f=0.5 and 1.0, are observed in the migration pattern for A4. If carry-over of substances from A1-A3, due to the overlap of GPC fractions, was expected or observed in the migration pattern for A5, then a migration pattern of TLC bands possessing these R_f values should also be observed for TLC fractionation of A4. A similar rationale was used for the TLC bands of B5 and B6 with the same R_f values as those found for B1-B3. That is, the very same R_f values should be observed in the TLC fractionation of B4. When comparing TLC bands of the same

R_f values between aspen and birch samples one might conclude that the substances present are the same or very similar. However, that conclusion is uncertain based on these data alone. The TLC bands with distinctly different R_f values support the contention for the existence of vegetation-specific organic compounds.

Table (2-2) summarizes the plate descriptions obtained under visible and UV light (366 nm) for the respective GPC fractions. The greatest concentration of material (based on band width and color intensity), in both aspen and birch samples, appeared to be located at R_f=0.3, 0.4, and 0.5 in the intermediate molecular weight GPC fractions 2, 3, and 4, respectively. Appearance of the plates under UV and visible light strongly indicated dissimilarities, between and within samples, of the lesser components (i.e. substances which do not represent the bulk of material applied to the plates based on band width and color intensity). For example, under UV light $B3_{1.0}$ exhibited a definite orange fluorescence, while $A3_{1.0}$ exhibited a faint yellow flourescence. Bands $A4_{1.0}$ and $A5_{1.0}$ were the only ones possessing a yellow-green fluorescence. The data in Table (2-2) show that the only TLC bands that appear to be the same, or very similar between forest types, are grouped in the following manner $B1_0$, $A1_0$; $B3_0$, $A3_0$; $B1_{0.2}$, $A1_{0.2}$; $B1_{1.0}$, $A1_{1.0}$;

Table 2-2. Plate descriptions of TLC I bands, under UV and visible light, that comprise each GPC fraction (A1 to A5 and B1 to B6).

	UV(366 nm)											Visible									
R_f	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	B6	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	B6
0	F YO	FY	-		-	M YO	-	-		-		DB	DB	B		DB	DB	B	B		B	
0.1		-			-	-	-	-			FO		B			LB	L YB	B	LB			DB
0.2	S YO					MO				FO		LB					YB				L YB	
0.3		S YO					S YO						DB					D YB				
0.4			S YO		SO			YO						DB		LY			DB			
0.5				S YO					YO						DB					DB		
0.6											MO											L YB
0.7																						
0.8								FY		FB									L YB	LY		
0.9		M PY	S PY		MB	FY	FB	SY			SB		L YB	LY		B	LY	LY	L YB			-
1.0	FB	SY	FY	S YG	S YG	FB	MY	MO	S PY	MB	SY	-	LB	LY	YB	BY	LY	LY	L YB	Y	Y	Y

Y=yellow
O=orange
B=blue
P=pale
F=faint
M=medium
S=strong
-=no fluorescence

B=brown
Y=yellow
D=dark
L=light
-=no apparent color

$B2_{0.1}$, $A2_{0.1}$; $B2_{0.3}$, $A2_{0.3}$; $B3_{0.4}$,$A3_{0.4}$; $B4_{0.5}$,$A4_{0.5}$; $B3_0$, $A3_0$; and $B5_0$, $A5_0$. All the other bands, 19 in birch and 12 in aspen, appear to be different, implying that distinctive substances comprised these zones of separation. In most cases fluorescence is not observed in materials with $R_f < 0.2$. Fluorescence may indicate the presence of conjugated double bonds in the organic material.

TLC Plate Descriptions (TLC II)

A second TLC procedure was employed, with limited success, to attempt a greater degree of separation than discussed previously. The method of sequential or step-wise development is rarely used and has an analogy to 2-dimensional TLC (Stahl, 1969) or to sample concentration using two solvents (Felton, 1981 in Lab Instrumentation: Chromatography Series III vol I). As carrier solvent migrated, the solute migrated with the solvent front as a narrow band 3-5 mm in width. The following plate descriptions (Table 2-3) indicated additional separations with very little tailing effect, implying a greater degree of separation. No R_f values are reported since they are not applicable. This procedure yields R_f values of 1.0 for each band. These data showed that as carrier solvent polarity increased, the apparent quantity of fluorescent material decreased, implying that the non-fluorescing substances were extremely polar.

Table 2-3. Plate Descriptions for TLC II bands observed for the fractionation of TLC I bands $A2_{0.3}$, $A3_{0.4}$, $A4_{0.5}$, $B2_{0.3}$, $B3_{0.4}$, and $B4_{0.5}$. Visualized under UV and visible light.

	UV						Vis					
Band	**$A2_{0.3}$**	**$B2_{0.3}$**	**$A3_{0.4}$**	**$B3_{0.4}$**	**$A4_{0.5}$**	**$B4_{0.5}$**	**$A2_{0.3}$**	**$B2_{0.3}$**	**$A3_{0.4}$**	**$B3_{0.4}$**	**$A4_{0.5}$**	**$B4_{0.5}$**
1	B	Y	FB	B	B	B	DY	LY	LY	-	Y	LY
2	Y	Y	B	FB	Y	Y	DY	LY	DY	Br	Y	LBr
3	O	-	YO	FY	Y	Y	LBr	Br	Br	DBr	DBr	LBr
4	YO	-	O	O	FB	FO	FY	DBr	Br	DBr	Br	LBr
5	FDO	O	O	-	O	-	FBr	Br	DBr	Br	Br	DBr
6	Y	-	-	-	FO	-	Br	Br	DBr	Br	Br	DBr

B=blue Br=brown O=orange Y=yellow F=faint D=dark L=light

It was stated earlier that, based on the data from TLC I, the principal bands of interest were $A2_{0.3}$, $B2_{0.3}$, $A3_{0.4}$, $B3_{0.4}$, $A4_{0.5}$, and $B4_{0.5}$. The R_f values and plate descriptions further implied that these bands appeared to be the same or very similar. They may be grouped in the following manner: $A2_{0.3} \cong B2_{0.3}$, $A3_{0.4} \cong B3_{0.4}$, and $A4_{0.5} \cong B4_{0.5}$. The data in Table (2-3) indicate, between the forest types, clear distinctions in the components that comprised these TLC I bands. The TLC II bands that exhibited the same or very similar characteristics between the vegetation types were: $A2_{0.3}2 \cong B2_{0.3}2$, $A2_{0.3}5 \cong B2_{0.3}5$, $A3_{0.4}4 \cong B3_{0.4}4$, $A3_{0.4}6 \cong B3_{0.4}6$, $A4_{0.5}1 \cong B4_{.5}1$, and $A4_{0.5}3 \cong B4_{0.5}3$.

Infrared Spectroscopy (TLC I)

All infrared spectra generally showed strong absorption in four principal regions(Figures 2-1 to 2-6, TLC I; Figures 2-7 to 2-12, TLC II). The broad band centered near 3400 cm^{-1} may be assigned to inter-molecular hydrogen bonded -OH. There are indications of undissociated -COOH present in all TLC fractions as reflected by very broad absorption from 3400-2500 cm^{-1} and absorption near 1720 cm^{-1} (Nakanishi and Solomon, 1977; Bellamy, 1975). Intensity of absorption due to -COOH and other carbonyl groups increased as the R_f of the TLC I fractions increased.

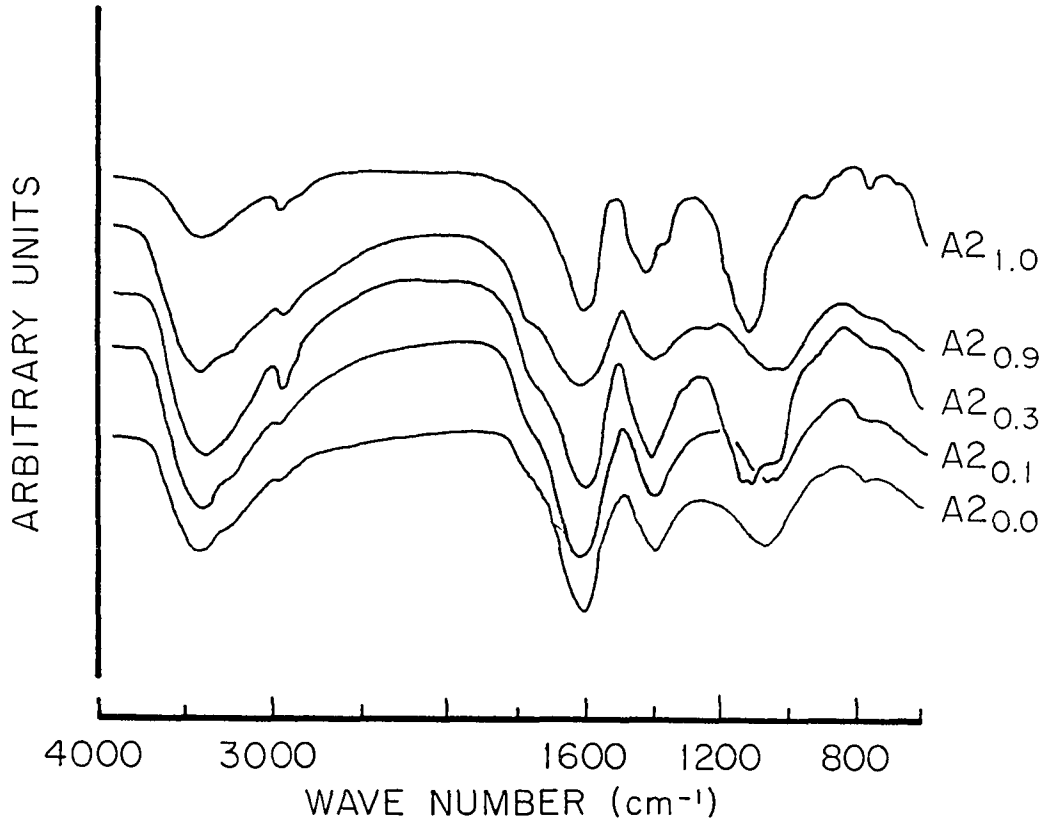

Figure 2-1. Infrared spectra of aspen extract TLC I bands $A2_0$, $A2_{0.1}$, $A2_{0.3}$, $A2_{0.9}$, and $A2_{1.0}$. All spectra were recorded as % transmittance versus wave number, grouped according to forest type, and placed in a single figure. This was done for figures 2-1 through 2-12.

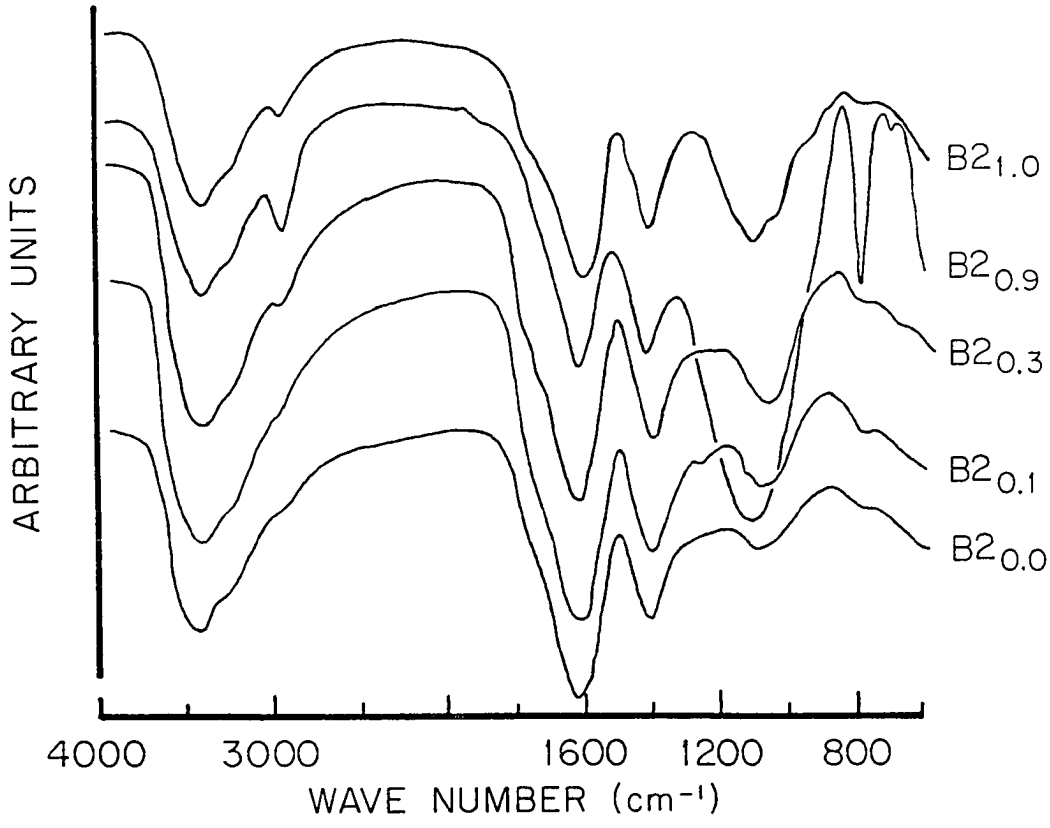

Figure 2-2. Infrared spectra of birch extract TLC I bands $B2_0$, $B2_{0.1}$, $B2_{0.3}$, $B2_{0.9}$, and $B2_{1.0}$.

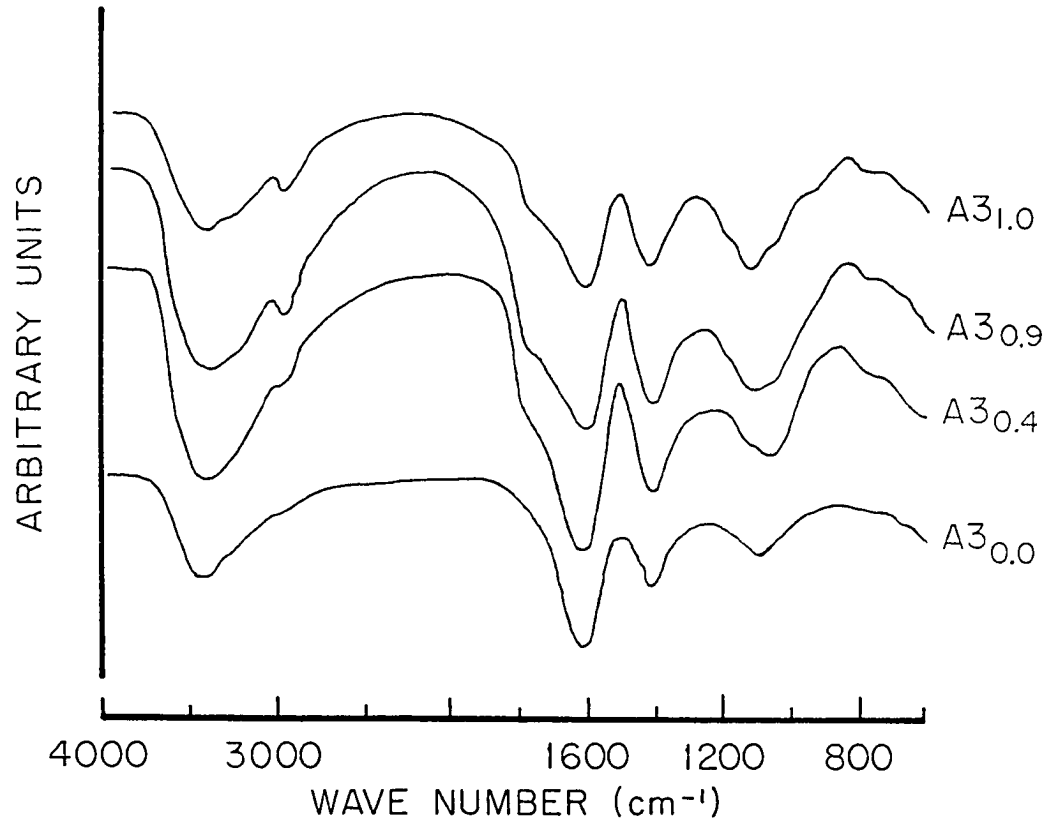

Figure 2-3. Infrared spectra of aspen extract TLC I bands $A3_0$, $A3_{0.4}$, $A3_{0.9}$, and $A3_{1.0}$.

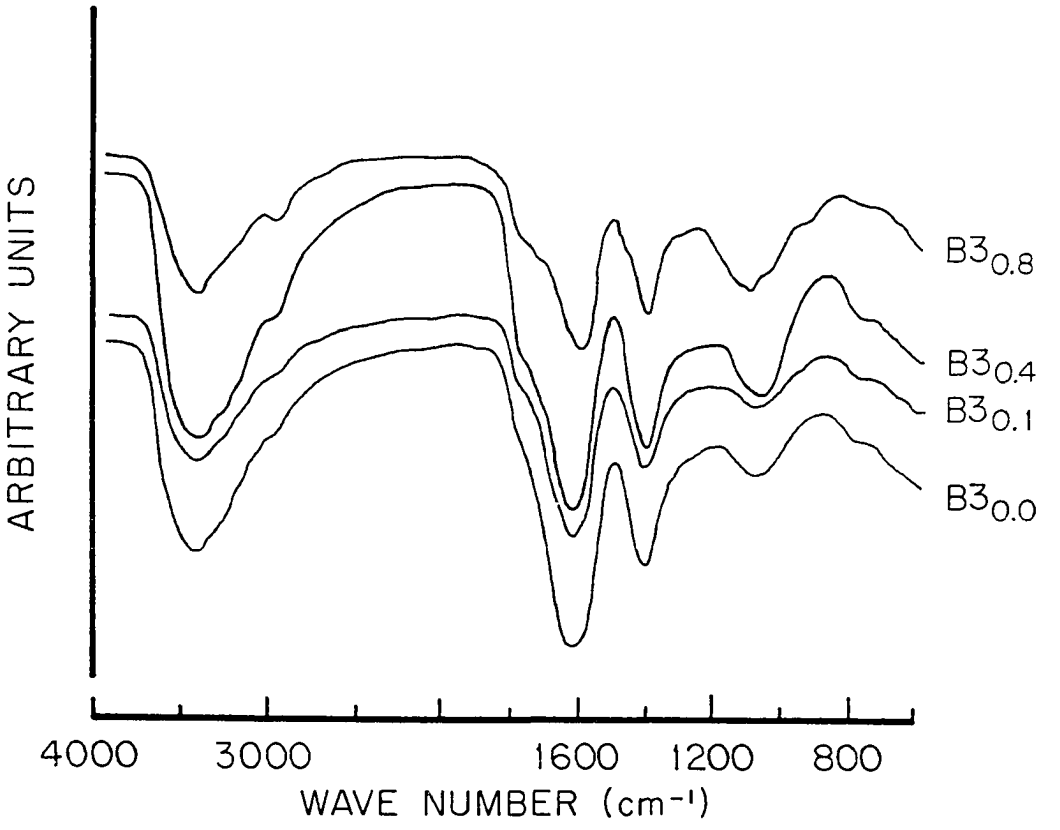

Figure 2-4. Infrared spectra of birch extract TLC I bands $B3_0$, $B3_{0.1}$, $B3_{0.4}$, and $B3_{0.8}$.

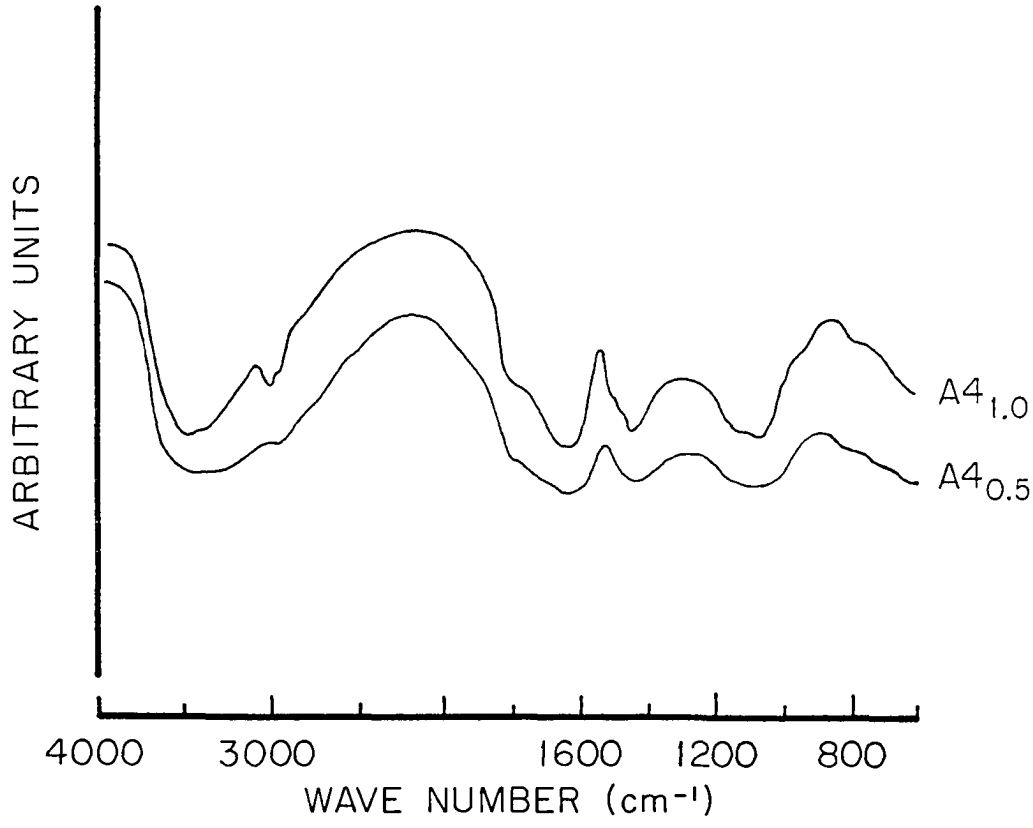

Figure 2-5. Infrared spectra of aspen extract TLC I bands $A4_{0.5}$ and $A4_{1.0}$

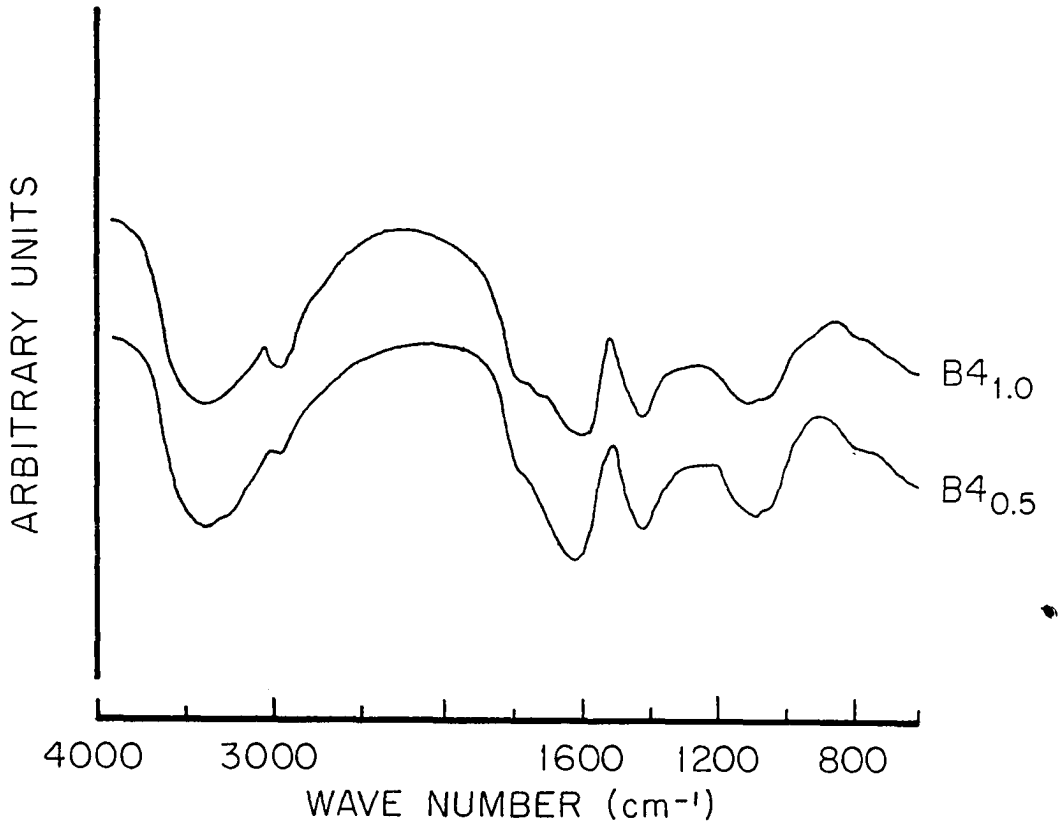

Figure 2-6. Infrared spectra of birch extract TLC I bands $B4_{0.5}$ and $B4_{1.0}$.

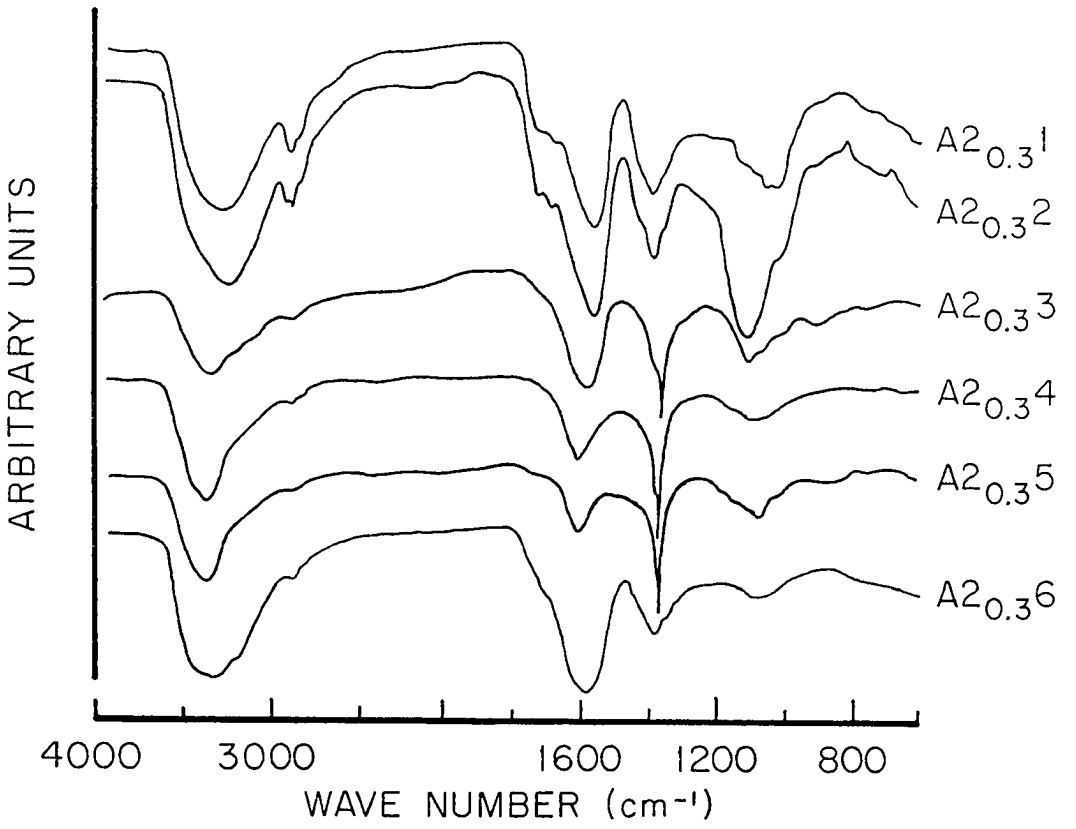

Figure 2-7. Infrared spectra of aspen extract TLC II bands $A2_{0.3}1$, $A2_{0.3}2$, $A2_{0.3}3$, $A2_{0.3}4$, $A2_{0.3}5$, and $A2_{0.3}6$.

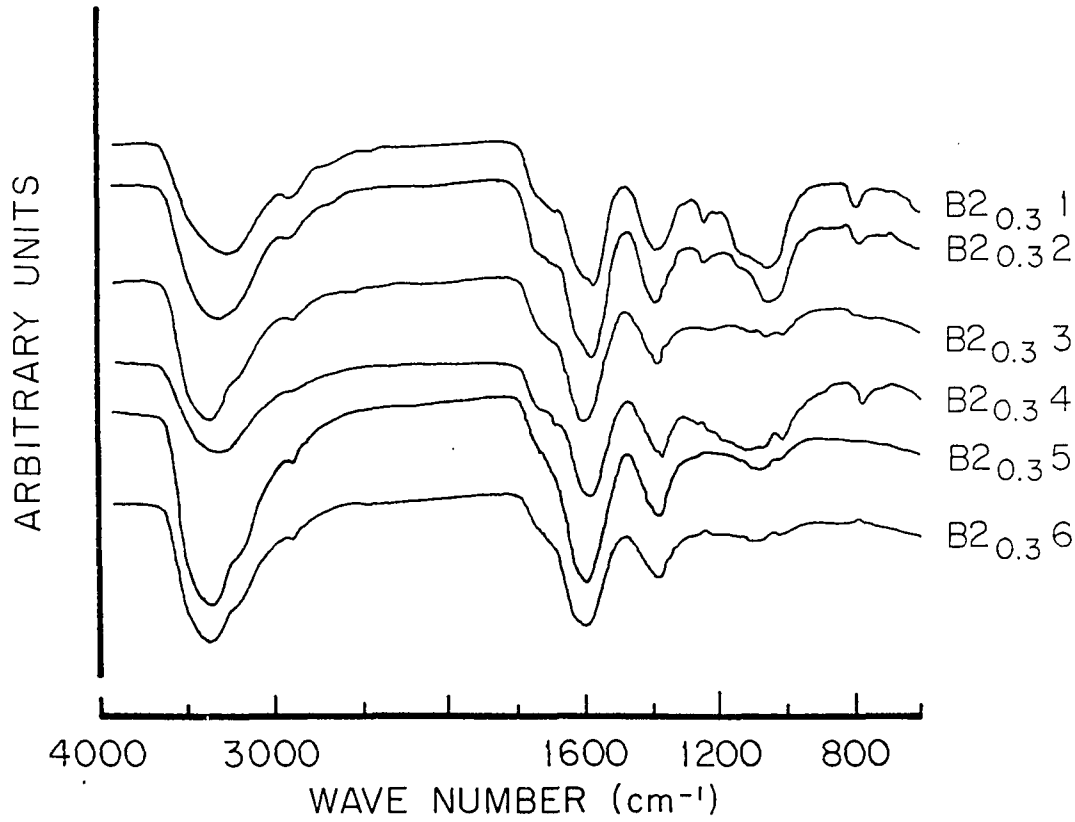

Figure 2-8. Infrared spectra of birch extract TLC II bands $B2_{0.3}2$, $B2_{0.3}2$, $B2_{0.3}3$, $B2_{0.3}4$, $B2_{0.3}5$, and $B2_{0.3}6$.

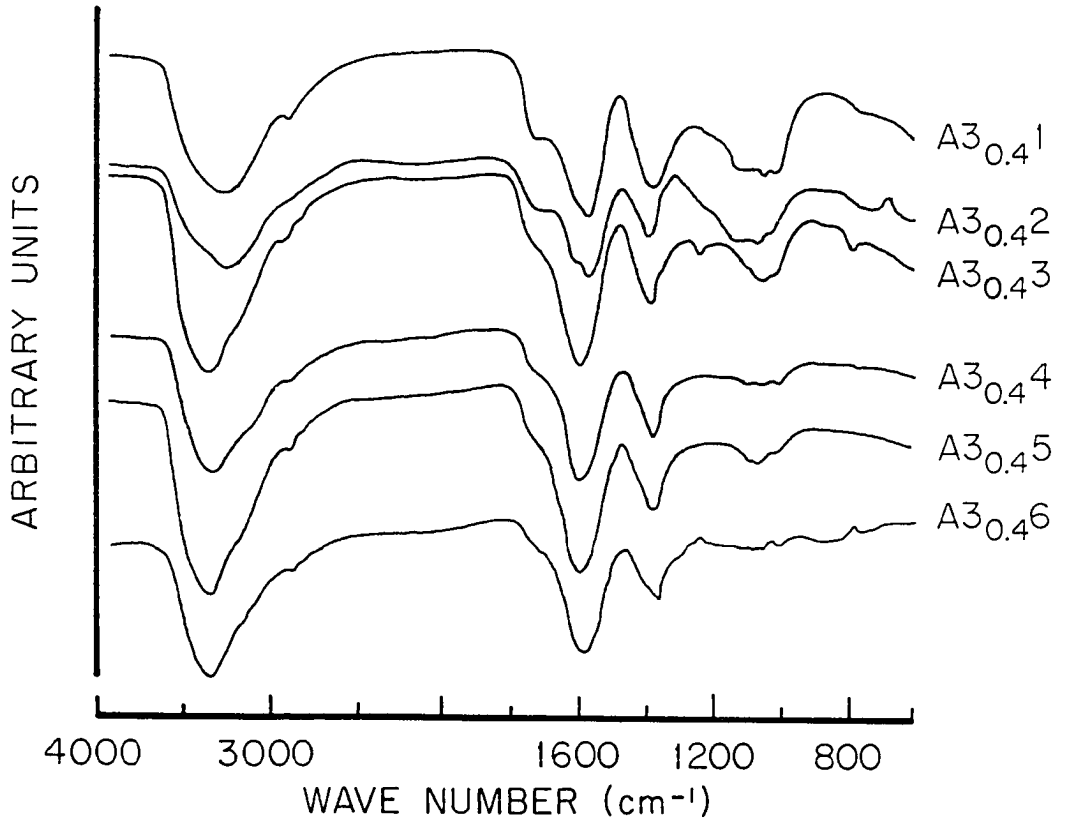

Figure 2-9. Infrared spectra of aspen extract TLC II bands $A3_{0.4}1$, $A3_{0.4}2$, $A3_{0.4}3$, $A3_{0.4}4$, $A3_{0.4}5$, and $A3_{0.4}6$.

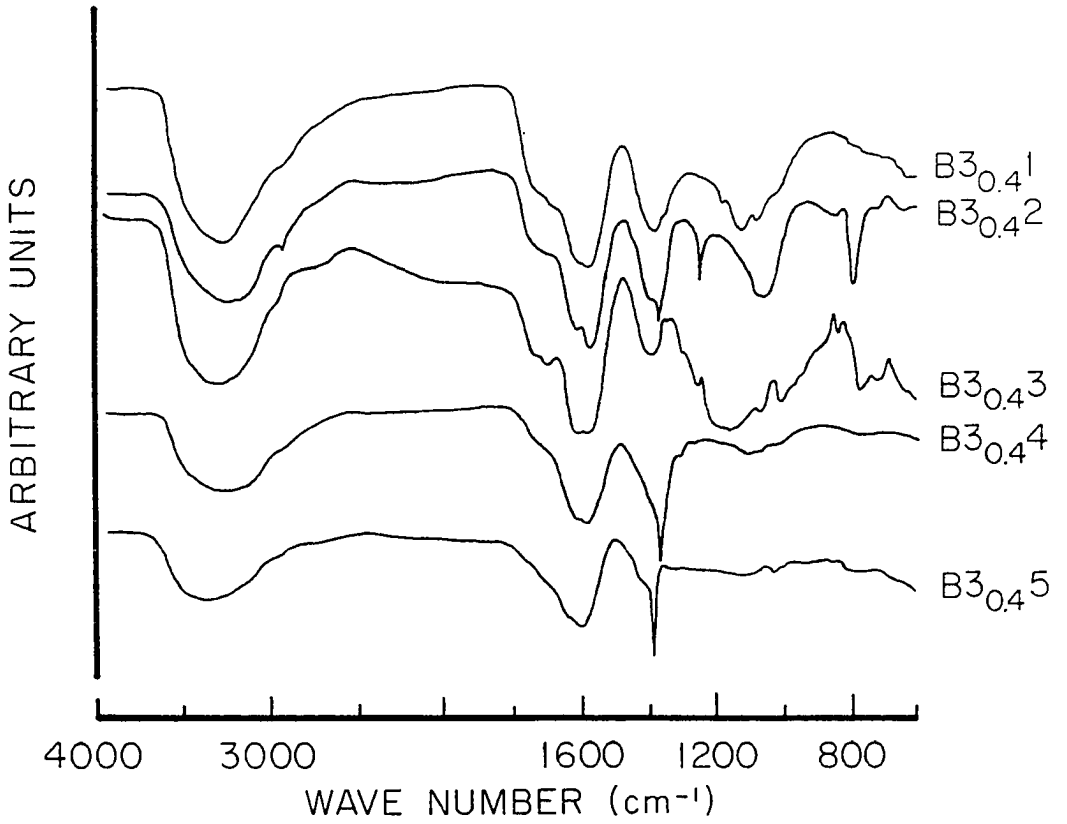

Figure 2-10. Infrared spectra of birch extract TLC II bands $B3_{0.4}1$, $B3_{0.4}2$, $B3_{0.4}3$, $B3_{0.4}4$, and $B3_{0.4}5$.

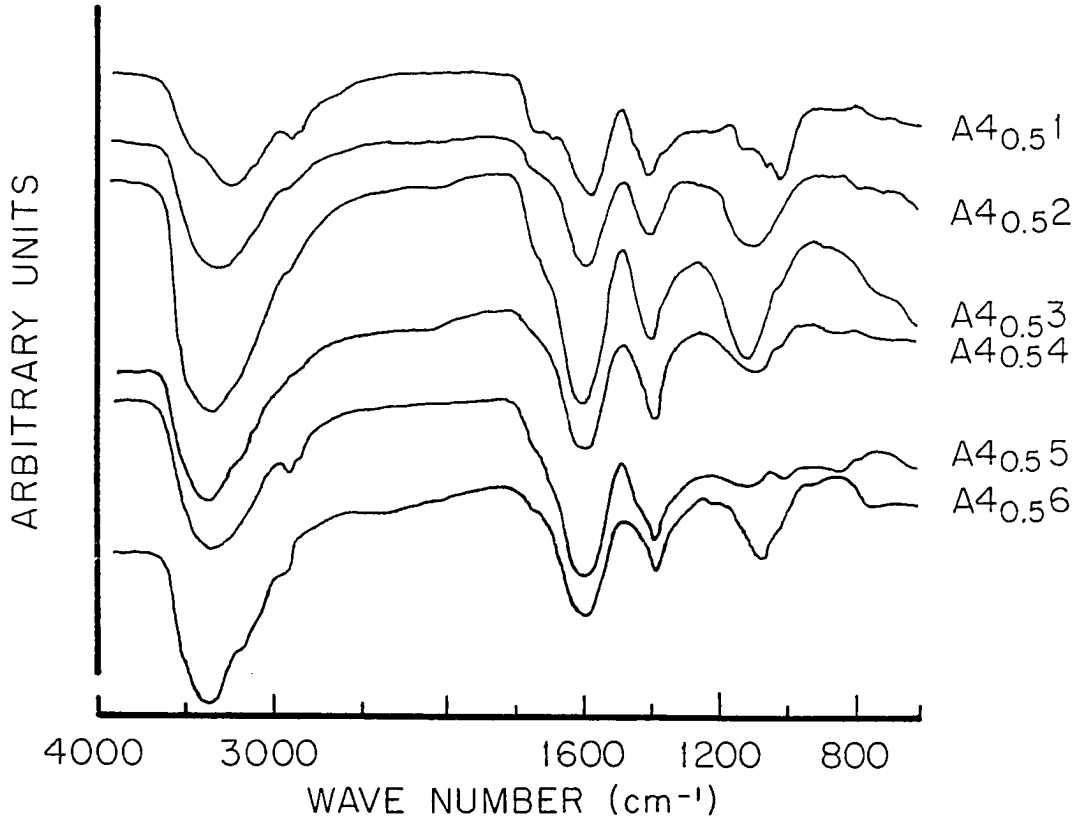

Figure 2-11. Infrared spectra of aspen extract TLC II bands $A4_{0.5}1$, $A4_{0.5}2$, $A4_{0.5}3$, $A4_{0.5}4$, $A4_{0.5}5$, and $A4_{0.5}6$.

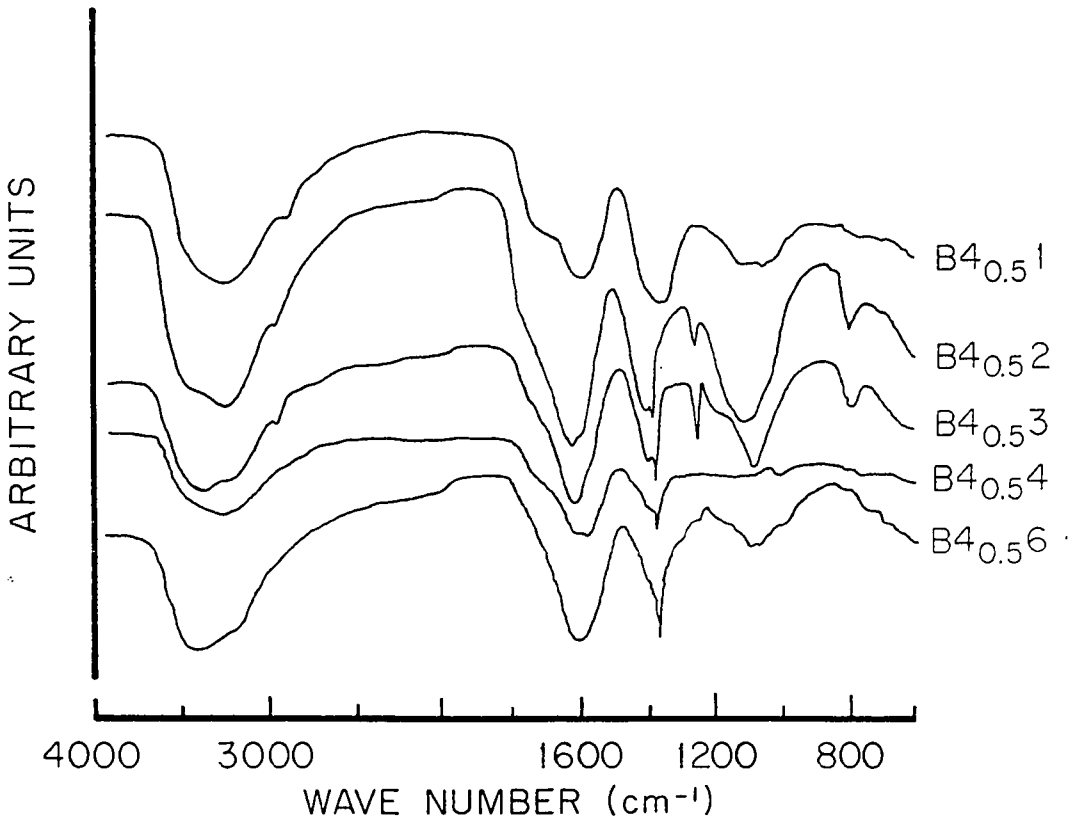

Figure 2-12. Infrared spectra of birch extract TLC II bands $B4_{0.5}1$, $B4_{0.5}2$, $B4_{0.5}3$, $B4_{0.5}4$, $B4_{0.5}5$, and $B4_{0.5}6$.

Generally, benzenoid compounds, alpha-beta unsaturated carboxylic acids, amides, and carboxylate anions give rise to strong infrared absorption from 1650-1590 cm^{-1} (Nakanishi and Solomon,1977; Bellamy, 1975). Aromatic substances that are unsymmetrically substituted and amides usually exhibit additional absorption near 1500 cm^{-1}. Absorption in this region was absent in all spectra, suggesting that these substances were not major contributors to overall structure of the material in the individual TLC fractions obtained by water extraction of mineral soil.

Strong absorption near 1630 cm^{-1} and 1400 cm^{-1} was probably due to the carboxylate anion and was present in all spectra (Nakanishi and Solomon, 1977; Bellamy, 1975). This result indicated metal-organic associations, possibly chelation or salts of these water soluble substances (Jackson and Hecky, 1980). Generally, the major differences of substances within forest types was reflected in the carbohydrate region, 1200-1000 cm^{-1}, of the spectra. Peak intensity in this region increased with increased R_f. Between forest types, the spectra did not reveal major differences in TLC I bands with equivalent R_f values.

Infrared Spectroscopy (TLC II)

The degree of polarity of substances in each TLC band is reflected in the spectra primarily in the aliphatic and

undissociated -COOH regions of the spectra (Figures 2-7 to 2-12). Generally as polarity increased, carbohydrate, alkyl and undissociated -COOH character diminished. A sharp peak near 1390 cm^{-1} was attributed to NO_3^- (Bellamy, 1975, Hempfling and Candler, 1986); it was more pronounced as polarity increased. Usually there was little or no strong evidence of NO_3^- in birch TLC II bands while NO_3^- character was most notable in comparable aspen TLC II bands. The reverse was true for birch TLC II bands exhibiting strong NO_3^- character. The other most notable difference in the spectra of comparable TLC II bands between forest types occurred in the carbohydrate region. This difference was evidenced by peak contours and intensities in this region (see Figures 2-7 to 2-12).

Conclusions

1. TLC may be used to further separate components comprising GPC fractions. Plate descriptions, including R_f values and visualization under UV and visible light, for TLC I indicated dissimilar components existed within and between soil extracts. Principal components from TLC I were contained in $A2_{0.3}$, $A3_{0.4}$, $A4_{0.5}$, $B2_{0.3}$, $B3_{0.4}$, and $B4_{0.5}$. This conclusion was based on band width and color intensity.

2. A second TLC procedure demonstrated that TLC I bands that appeared to be the same or similar could be further fractionated. Distinct differences were observed in the TLC II bands, within and between forest types, as reflected by the TLC II plate descriptions and infrared spectra.
3. Infrared spectra revealed little detailed structural information about proposed ligands. Metal-organic associations, possibly chelates, were indicated by the presence of carboxylate anions causing absorptions near 1630 and 1400 cm^{-1}.

Chapter 3

Characterization of Complexation by Water Soluble Humic Substances Using Ion-Exchange Chromatography

Introduction

Ion exchange chromatography offers a rapid and elegant method for the study of strongly complexed metals in natural systems. Variations of the method have been used to investigate complexes of Zn, Cd, Cu, Ni, Pb, and Ca (Jones and Manahan, 1975; Stolzberg and Rosin, 1977; Mackey, 1983; Hendrickson and Corey, 1983; and, Sunda 1984). Most of these studies have been directed towards examination of complexation by organic substances in aquatic systems. No attempts were made to separate individual organic components that strongly complexed metals. Therefore, metals that were found to be strongly bound represented an overall complexation by a potentially wide variety of natural chelating agents, some of which may not have strongly bound metals.

Hendrickson and Corey (1983) determined that this method was applicable to the study of metal complexation by natural chelating agents extracted from sewage sludge-ammended soils. Kaurichev et al., (1977), in a novel approach, used an ion exchange resin as a thin-layer chromatographic sorbent. They found that three distinct iron complexes were formed by organic substances in a

water extract from the A_1 horizon of a Sod-Podzolic soil. One band exhibited chromatographic characteristics similar to Fe-citrate complexes. The other two bands, because of their chromatographic behavior, demonstrated the strength of iron complexation to be greater than Fe-citrate complexes but less than Fe-EDTA.

The two previous chapters of this thesis have demonstrated the complex nature of aqueous extracts from soils. Many apparently distinct components possessing the ability to chelate metals may be obtained from a single extract. This chapter describes the application of ion exchange chromatography (after Stolzberg and Rosin, 1977) to determine which TLC bands obtained in the present study strongly complex iron and copper. This variation of the ion exchange chromatography technique is particularly well suited for studies on trace quantities of metals, such as copper and iron, that readily form strong complexes with water soluble humic substances. The results presented here demonstrate that differences in chelates exist. These results strongly support the central hypothesis which was that forest-type-specific suites of water soluble organic compounds capable of chelating metals may be, in part, responsible for observed differences in rates of element cycling in aspen and birch forests.

Methods and Materials

Ion Exchange Chromatography

Chelex 100 (sodium form) is a strong chelating resin. Metals that are uncomplexed or weakly complexed are readily bound by the resin. Strongly complexed copper or iron should elute from the resin bed in a nearly quantitive manner.

The solutions representing individual TLC I and TLC II bands (see chapter 2, Methods and Materials) were split into one 5 ml and two 10 ml aliquots. The 10-ml portions of each solution were separately passed through 10 X 0.5 cm columns containing 2.5 cm Chelex 100 (after Stolzberg and Rosin, 1977). Five milliliters of each effluent were separately collected for subsequent atomic absorption spectrometry (AAS). The 5-ml aliquot of original, untreated solution, designated NC (no chelex treatment) was also set aside for atomic absorption spectrometry (AAS). The percent yield of the respective metals (Fe and Cu) was determined as % Yield = (ppm of effluent from Chelex 100)/(ppm of NC). Metals were considered strongly bound when % Yield = 80% or greater (Stolzberg and Rosin, 1977).

All TLC bands from the first thin-layer procedure (TLC I) were designated as: Forest type (A = aspen and B = birch), GPC fraction R_f, e.g. $B1_{0.2}$ = birch GPC fraction 1, R_f=0.2. All TLC bands from the second TLC

procedure (TLC II) are designated as: Forest type, GPC fraction R_f, band number, e.g. $B3_{0.4}1$= birch GPC fraction 3, R_f=0.4, TLC II band number 1.

Atomic Absorption Spectrometry

All metal concentration determinations were carried out on a Perkin-Elmer model 5000 Atomic Absorption Spectrophotometer equipped with a graphite furnace, model HGA-500, and an autosampler, model AS-40.

Results and Discussion

Ion Exchange Chromatography

1983 Samples--TLC I

Preliminary investigations regarding metal distributions and metal binding by organic materials (Candler, 1985b) indicated that Mn was not strongly bound. This result was not surprising due to the chemical nature of Mn. Gamble et al. (1976) determined that Mn^{2+} was weakly bound to fulvic acid donor groups. The species, $Mn(OH_2)_6^{2+}$, was bound electrostatically and by hydrogen bonding. The most important and stable oxidation state for manganese is Mn^{2+}. This species, in neutral or acidic aqueous solutions, exists as the hexaquo ion and is very resistant to oxidation. $Mn(OH)_2$ is formed in basic media and is readily oxidized by air to manganese oxides. Chelates and other complexes of manganese in higher oxidation states are known but these are generally unstable in aqueous solutions. The ligands are easily

replaced by water, and manganese in these higher oxidation states is easily reduced. Copper and iron, on the other hand, readily form stable complexes in aqueous solutions. Iron (III) and copper (II) are the most stable oxidation states for these two elements. Further oxidation by air is not thermodynamically favorable. Iron III also forms a hexaquo ion in aqueous solutions which readily hydrolyzes to ferric hydroxide. In basic media, ferric hydroxide acts as an acid and is preciptated as hydrous ferric oxide. In the presence of ligands containing oxygen, water is readily displaced from the coordination sphere of iron (III), and they form complexes or chelates. Similarly, water molecules are readily displaced from the coordination sphere of copper (II) by organic ligands to form very stable complexes (Cotton and Wilkinson, 1967).

The quantity of metals removed from the plates, determined as the sum of ppm X 25 ml of all NC treatments, should not exceed the quantity placed on the plates. Some discrepancies between amounts placed on and amounts eluted off the plates were observed upon determination of mass balances for Cu, Fe, and Mn (Table 3-1). In Table 3-1, the amount of metal eluted from the plate is put in italics where it exceeds the amount applied to the plate by ten percent. An error of ± 10% was deemed acceptable for this procedure. These discrepancies were more

Table 3-1. Mass balance for Cu, Fe, and Mn (in µg) for the TLC I procedure (1983 samples)

Sample	plate	Cu on	Cu off	Fe on	Fe off	Mn on	Mn off
A1	1	2.3	2.0	34.0	33.6	0.7	*1.7*
	2	2.5	2.8	31.1	32.0	0.6	*1.3*
	3	2.2	2.2	26.9	23.5	0.6	0.6
	4	2.2	*5.6*	26.9	22.7	0.6	0.6
A2	1	9.5	9.4	73.5	*183.9*	0.7	*2.2*
	2	7.8	7.6	60.8	*198.1*	0.6	*1.8*
	3	10.3	10.4	79.9	*163.9*	0.7	*10.7*
	4	10.7	10.4	83.1	*175.2*	0.8	*8.0*
A3	1	7.3	5.2	76.9	78.9	9.6	2.8
	2	4.8	5.1	50.5	55.7	6.3	0.8
	3	7.3	9.7	76.9	78.8	9.6	4.5
	4	9.0	9.6	94.5	97.3	11.8	5.7
A4	1	8.4	8.2	14.7	*69.3*	78.1	4.3
	2	6.7	6.7	11.6	*229.4*	61.9	5.9
	3	7.6	7.8	13.2	*106.3*	70.0	67.8
	4	9.0	9.0	15.7	*43.0*	83.5	18.9
A5	1	2.6	*4.3*	10.7	*14.4*	22.5	1.8
	2	2.6	*3.9*	10.7	*19.8*	22.5	2.5
	3	2.6	*5.3*	10.7	9.2	22.5	4.8
	4	2.6	*6.4*	10.7	8.4	22.5	6.5
B1	1	1.9	1.7	25.3	15.1	1.3	0.8
	2	1.9	1.2	25.3	20.2	1.3	1.2
	3	2.2	2.4	30.4	31.4	1.5	0.9
	4	2.8	2.8	38.0	41.7	1.9	0.9
B2	1	6.4	6.2	80.4	55.7	2.0	2.4
	2	6.4	5.3	80.4	80.2	2.0	1.6
	3	6.2	6.2	77.2	63.5	1.9	0.9
	4	5.1	5.5	64.3	59.4	1.6	1.2
B3	1	11.7	11.8	90.0	*377.5*	18.0	18.0
	2	10.1	10.1	79.1	*245.2*	15.8	7.2
	3	9.9	9.9	76.3	*177.4*	10.4	*28.0*
	4	6.7	6.6	51.8	*207.4*	10.4	*27.9*
B4	1	6.8	6.8	22.3	*79.7*	12.7	6.8
	2	6.1	6.2	20.2	*151.1*	11.5	5.2
	3	9.4	9.4	*30.8*	*52.7*	17.6	8.3
	4	10.7	10.5	*35.1*	*54.4*	20.0	11.7
B5	1	1.7	1.7	30.1	3.8	4.5	0.9
	2	1.3	1.2	22.6	8.3	3.4	0.4
	3	1.7	1.6	30.1	9.2	4.5	0.3
	4	1.4	1.5	25.6	7.8	3.8	0.2
B6	1	4.8	*6.0*	29.7	23.3	12.2	2.6
	2	4.8	4.6	29.7	*62.2*	12.2	2.9
	3	4.8	*11.7*	29.7	24.1	12.2	9.2
	4	lost----------					

pronounced for Fe than Cu and Mn. For the most part, the quantity of Cu placed on the plates was approximately equal to the quantity of Cu eluted from the plates, whereas the amount of Mn removed was usually lower than Mn applied to the plates. This result indicated that Mn was retained by the sorbent. Most of the Cu, Fe, and Mn were found in single TLC fractions $A2_{0.3}$, $B2_{0.3}$, $A3_{0.4}$, $B3_{0.4}$, $A4_{0.5}$, and $B4_{0.5}$, in agreement with the results of previous studies (Candler, 1985b).

Due to the amount of sample handling some environmental contamination was possible. This contamination was particularly likely where serial dilutions of original solutions of the TLC I bands were performed. Serial dilutions were sometimes necessary since metal concentration ranges were difficult to assess and only small quantities of original material were available for analysis. Dilutions in these cases amounted to 25-100 fold. Blanks for Cu, based on water extracts of ten plates, yielded a range of 0 ppm (not detected = ND) -0.002 ppm, Mn--ND, and Fe ND-.005 ppm. A nitric acid digest of four blank prewashed plates revealed that there was 0.512 µg Fe/mg sorbent which was not extractable with water. Organic compounds known to chelate iron, such as malonic acid or EDTA, did remove Fe from the sorbent. As well, organics from soil extracts capable of chelation could remove Fe from the sorbent.

Four test plates were used to determine the ability of malonic acid and EDTA to extract Fe from the sorbent. EDTA removed an average of 40% of the Fe whereas malonic acid removed an average of about 72% of the Fe. In the case of B3 plate #1, 782.8 µg Fe is available as background (based on the quantity of sorbent scraped from the plate). Ninety µg of sample Fe was placed on the plate. Altogether, 782+90=872 µg Fe could possibly be eluted off the plate, but only 377.5 µg were recovered from the plate. If it is assumed the original 90 µg eluted, then 377.5-90=287.5 µg additional Fe was removed from the plate. This result suggested that all chelation sites of the organic substances were not occupied in the original unknown sample, and that these sites were binding Fe derived from the plate. Where mass balances match, or nearly do, one can conjecture that available chelating sites are already occupied. If the excesses are consistent then it might be possible to determine if background Fe was extracted by the organic material placed on the plates. However, since the excesses were not consistent, external contamination or a combination of external contamination and extracted background Fe must be considered. This consideration does not necessarily negate the present chelation studies for iron and copper. In fact, if background Fe was removed by the organics in these TLC bands then chelation is implicated.

Solutions of each TLC band were subjected to a variation on the method of Stolzberg and Rosin (1977), which required a molar excess of metal added before chromatography, to determine if Fe, Cu, and Mn were strongly bound. Figures 3-1 to 3-6 depict percent yield of metal after this treatment (% yield = 80% implied that metals were strongly bound). TLC bands with the same R_f may contain the same or similar substances, as noted earlier. As a test of the central hypothesis, statistical tests were employed to determine metal binding distinctions within and between vegetation-type derived components. Statistical tests were applied to Cu and Fe only since Mn was not strongly bound. The individual fractions were used as the replicates and were considered representative of the substances in the aspen and birch soils studied because each pooled extract was obtained from many individual extracts. For this reason the differences observed within and between the forest-type extracts are considered valid, but the experimental design does not consider field biological variations.

Bartlett's test for homogeneity of variances in the percent yields of Cu and Fe demonstrated that these data were heteroscedastic. Several attempts using various transformations to homogenize the variances were unsuccessful and precluded the use of parametric statistical comparisons. Therefore, non-parmetric statistical tests were used for pertinent comparisons.

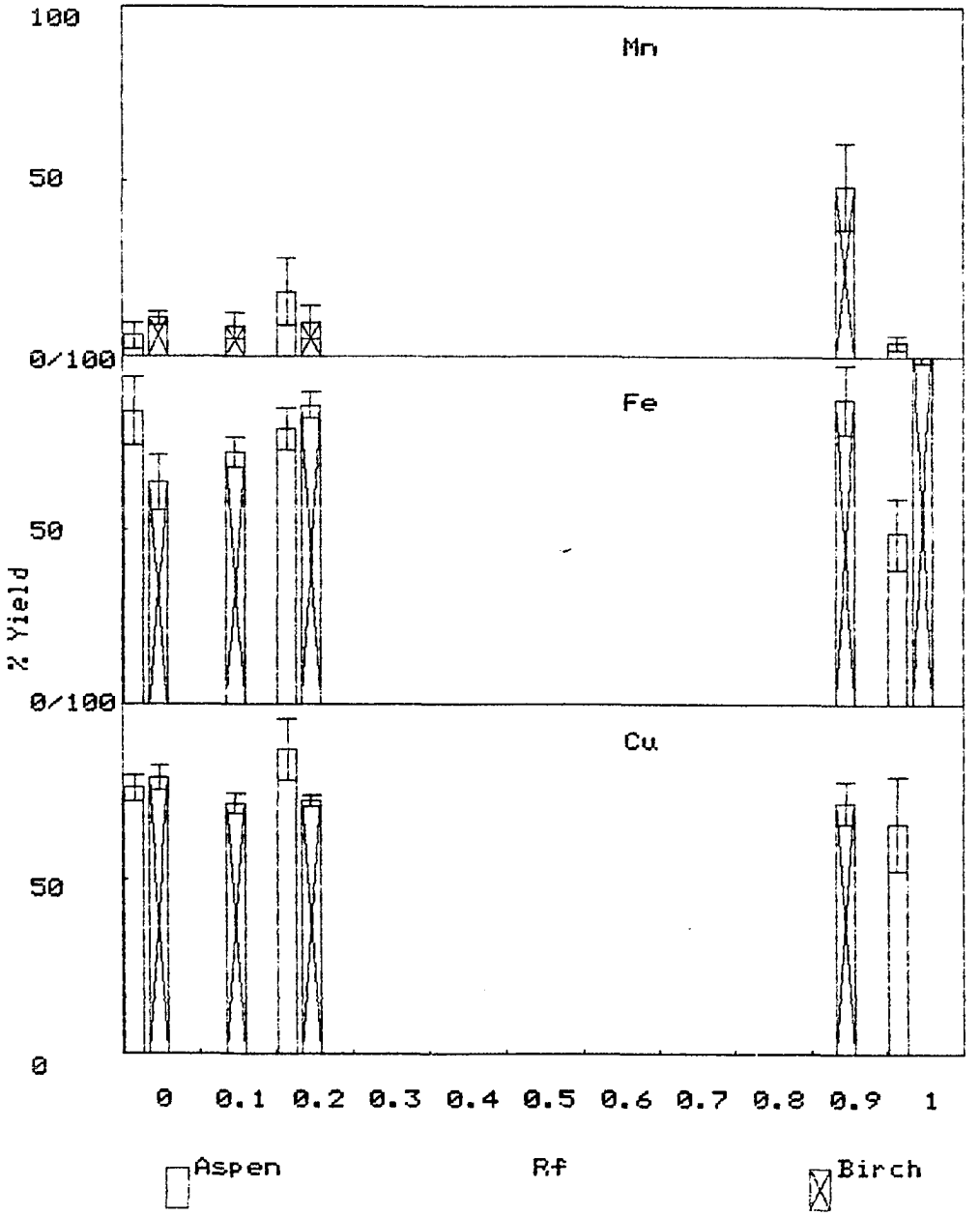

Figure 3-1. Percent yield of manganese, iron, and copper for each TLC I band in GPC fraction 1 (K_d=0) from aspen and birch soil extracts. 1983 samples (n=8: four replicate plates, two determinations per TLC I band).

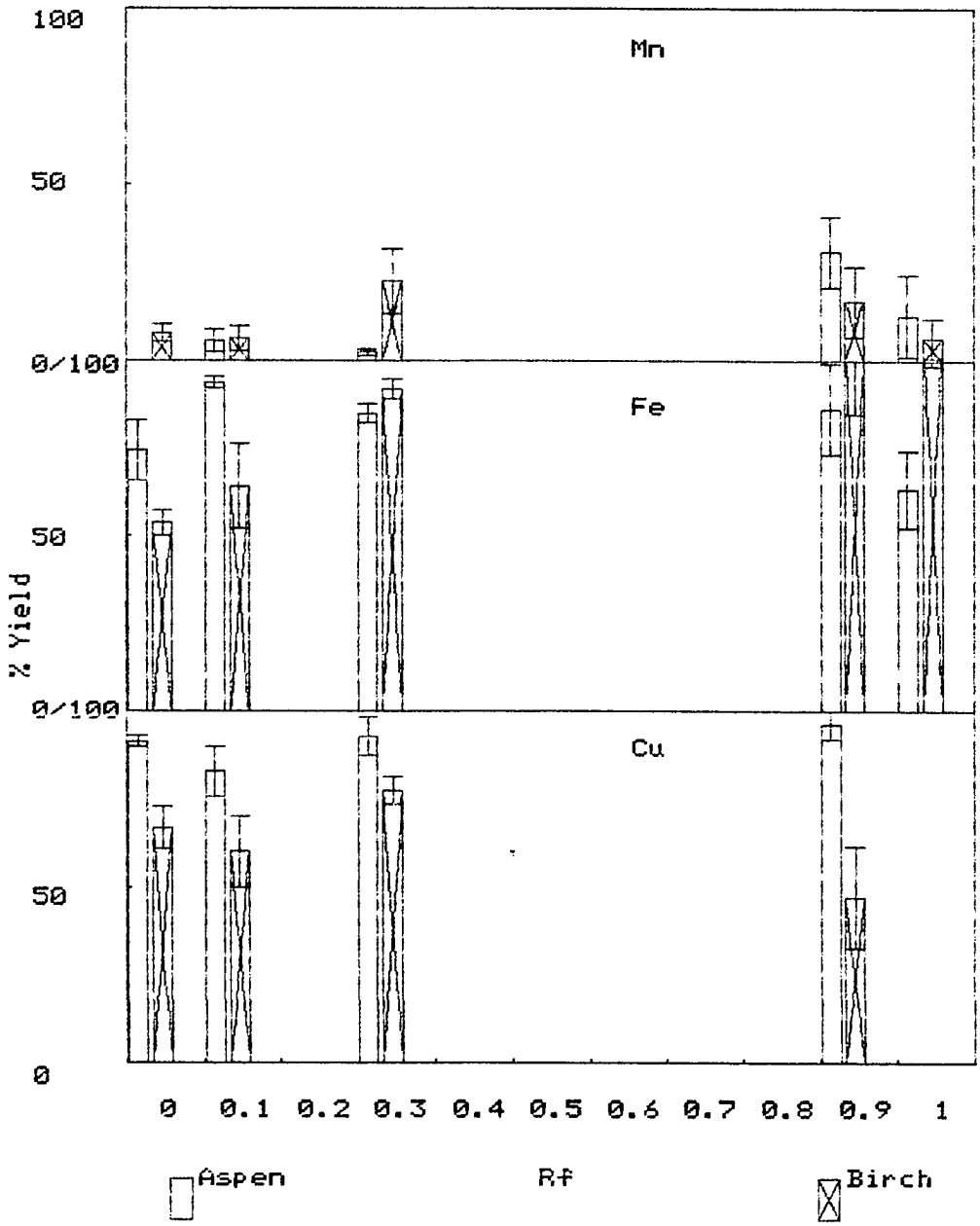

Figure 3-2. Percent yield of manganese, iron, and copper for each TLC I band in GPC fraction 2 (K_d=0.2) from aspen and birch soil extracts. 1983 samples (n=8: four replicate plates, two determinations per TLC band).

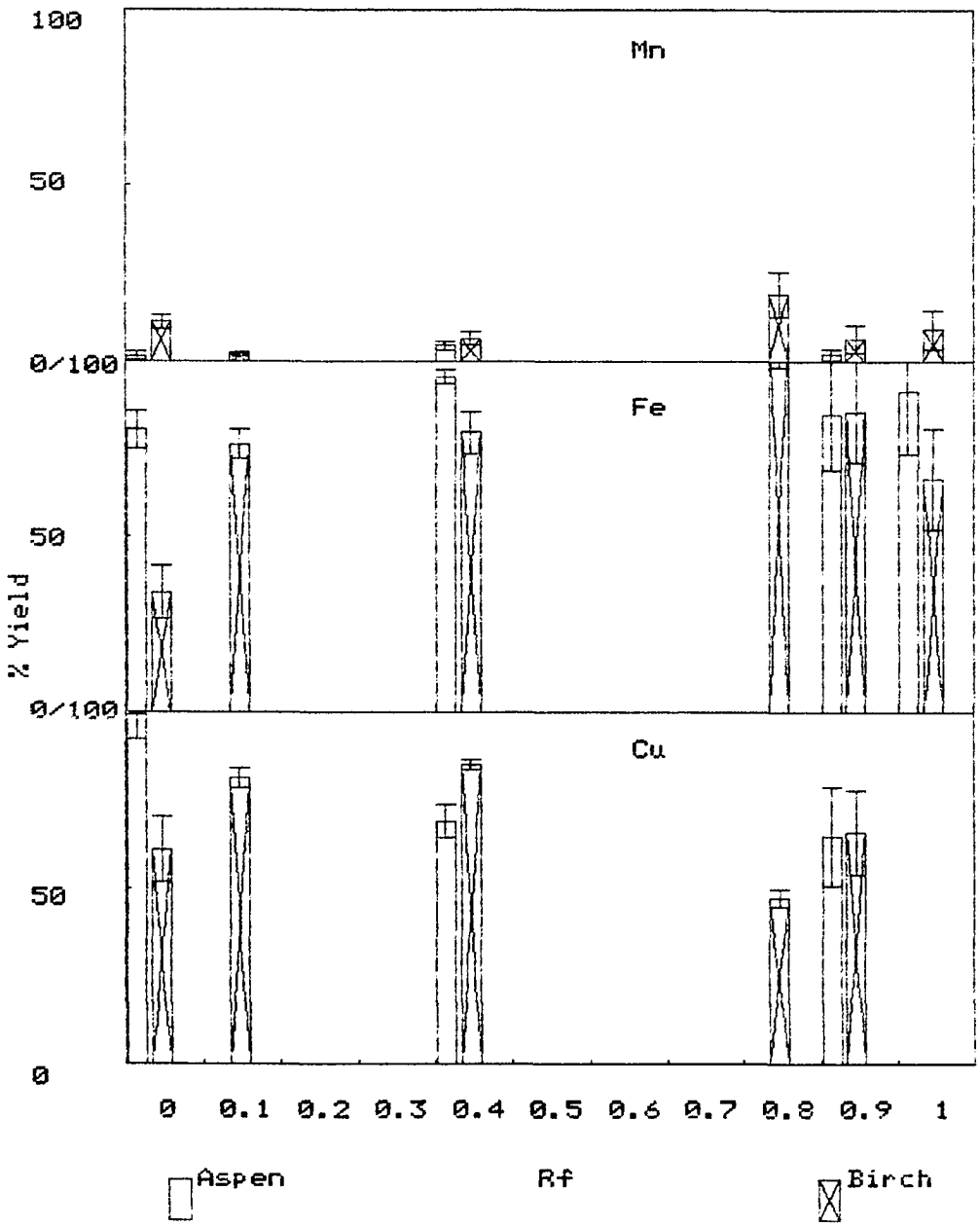

Figure 3-3. Percent yield of manganese, iron, and copper for each TLC I band in GPC fraction 3 (K_d=0.4) from aspen and birch soil extracts. 1983 samples (n=8: four replicate plates, two determinations per TLC band).

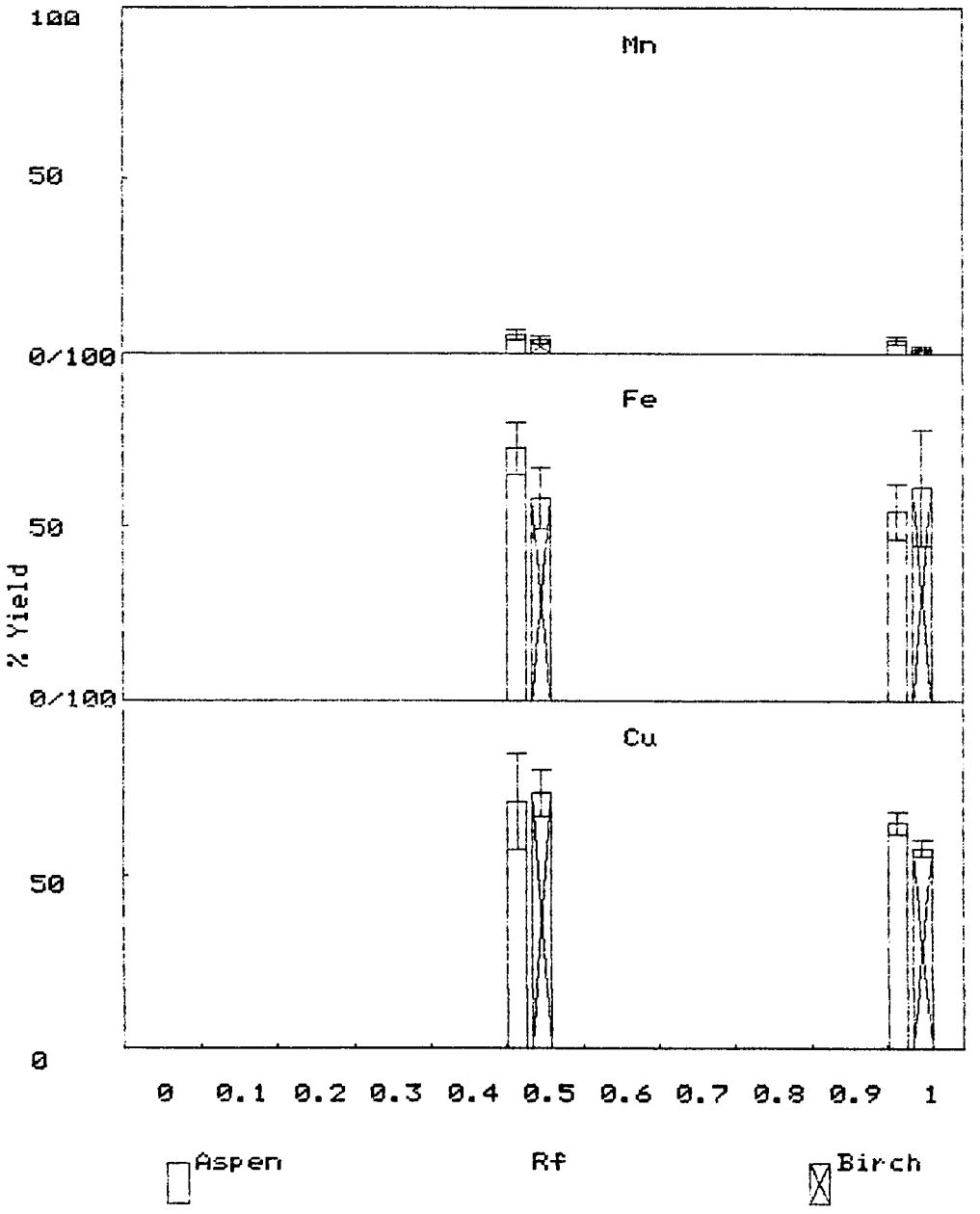

Figure 3-4. Percent yield of manganese, iron, and copper for each TLC I band in GPC fraction 4 (K_d=0.6) from aspen and birch soil extracts. 1983 samples (n=8: four replicate plates, two determinations per TLC band).

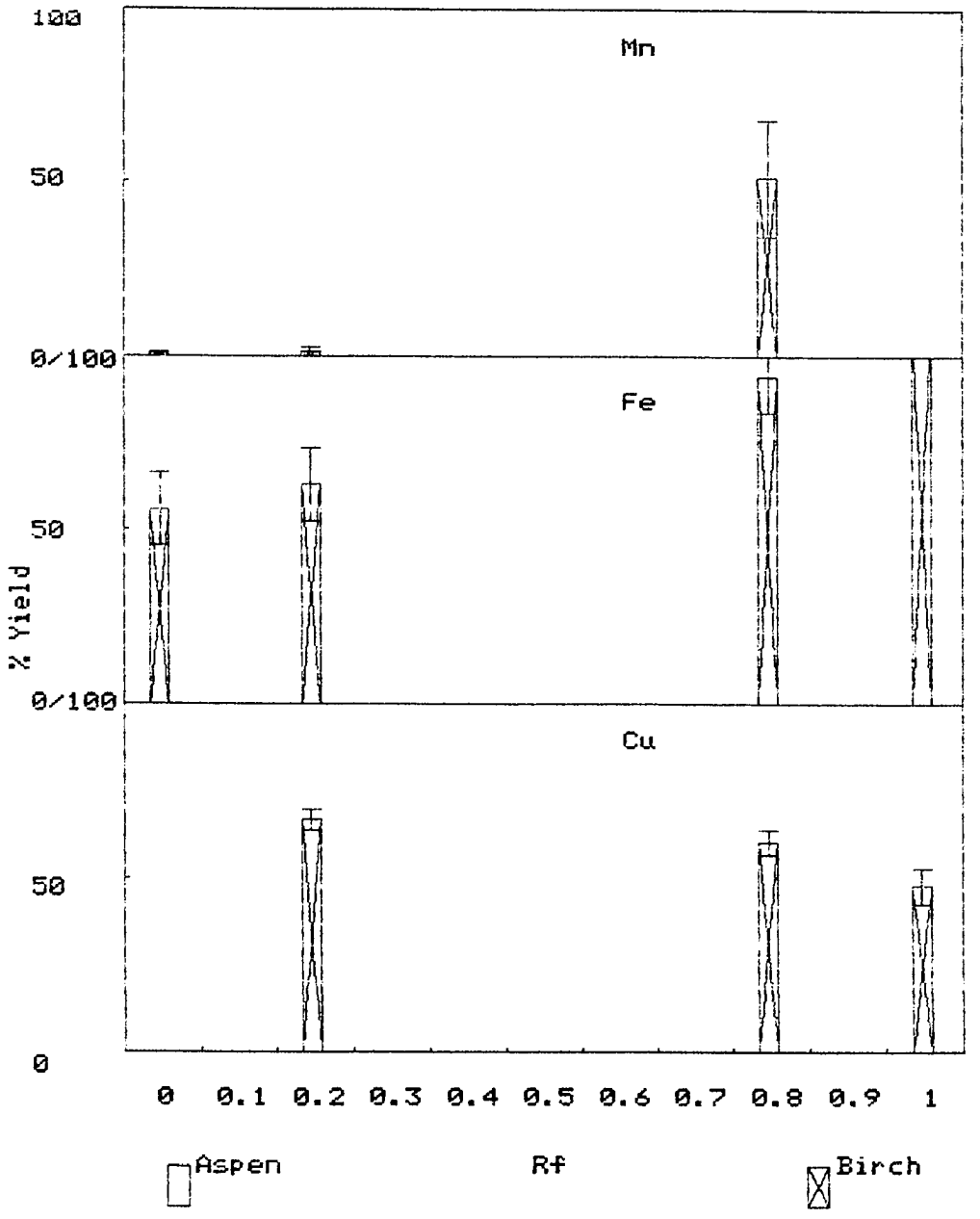

Figure 3-5. Percent yield of manganese, iron, and copper for each TLC I band in GPC fraction 5 (K_d=0.75) from birch soil extracts. 1983 samples (n=8: four replicate plates, two determinations per TLC band).

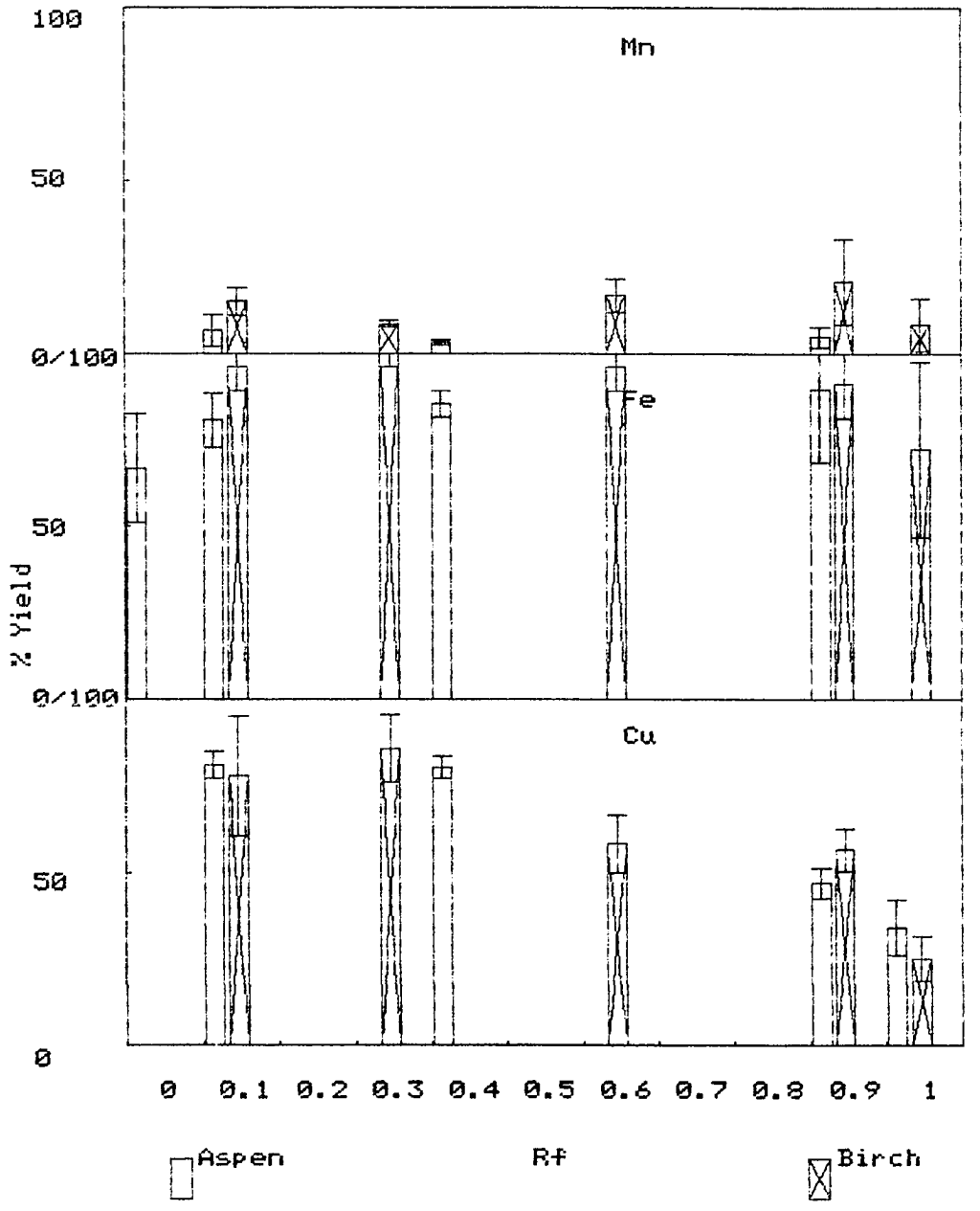

Figure 3-6. Percent yield of manganese, iron, and copper for each TLC I band in aspen GPC fraction 5 (K_d=0.8) and birch GPC fraction 6 (K_d=0.8). 1983 samples (aspen n=8: four replicate plates, two determinations per TLC band--birch n=6: three replicate plates, two determinations per TLC band).

These included the Kruskal-Wallis and Mann-Whitney tests. These comparisons are analogous to the parametric one-way ANOVA and Student's t tests, respectively.

Overall percent yield for Cu (birch) = 64.9 and Cu (aspen) = 73.6 indicated that Cu was not strongly bound by organics in either forest-type extract. However, results of the Mann-Whitney test for these averages showed a significantly greater copper binding ability in aspen than in birch ($z = 3.2$, $\alpha = 0.0014$). Iron on the other hand was equally strongly bound by both forest-type extracts (Mann-Whitney test $z = 0.448$, percent yield Fe (aspen) = 78.9, and percent yield Fe (birch) = 80.2). Significantly more iron than copper was chelated in both vegetation types. Table 3-2 summarizes the average percent yield of copper and iron in each TLC fraction. Ten fractions in aspen bound copper strongly whereas there were only seven fractions in birch. Seventeen fractions in birch and 15 in aspen appeared to strongly bind iron.

The Kruskal-Wallis test was applied to the data to determine where significant differences occurred among TLC bands, within a given GPC fraction, within a vegetation type. Finally, bands with the same R_f that appeared to bind metals strongly were tested to determine which vegetation type bound metals most effectively. The results of the Kruskal-Wallis test

Table 3-2. Average percent yield of Cu and Fe ± the standard error (SE) for each TLC I band for the 1983 aspen and birch samples (four replicate plates with two % yield determinations).

TLC Fraction	% yield Cu	SE	% yield Fe	SE	TLC Fraction	% yield Cu	SE	% yield Fe	SE
$A1_0$	75.9	3.6	84.1	9.8	$B1_0$	78.8	3.5	63.6	7.9
$A1_{0.2}$	87.0	8.9	78.9	6.0	$B1_{0.1}$	71.4	2.9	72.2	4.3
$A1_{1.0}$	65.8	13.6	49.1	10.0	$B1_{0.2}$	72.5	1.6	85.8	3.8
					$B1_{0.9}$	71.9	6.0	87.5	10.0
					$B1_{1.0}$	ND		110.6	12.5
$A2_0$	91.4	1.6	74.3	8.5	$B2_0$	66.8	6.0	53.8	3.7
$A2_{0.1}$	82.8	7.2	93.6	1.5	$B2_{0.1}$	60.0	10.0	64.2	12.1
$A2_{0.3}$	92.7	5.5	84.8	2.7	$B2_{0.3}$	77.5	3.9	91.7	2.8
$A2_{0.9}$	96.2	4.2	86.1	12.9	$B2_{0.9}$	47.0	14.4	103.7	19.2
$A2_{1.0}$	ND		63.5	10.9	$B2_{1.0}$	ND		108.8	10.2
$A3_0$	99.3	7.0	80.4	5.4	$B3_0$	60.9	9.4	34.2	7.6
$A3_{0.4}$	69.1	4.8	95.4	1.9	$B3_{0.1}$	81.4	2.8	76.3	4.2
$A3_{0.9}$	64.8	14.1	84.5	15.4	$B3_{0.4}$	85.3	1.5	79.6	5.8
$A3_{1.0}$	ND		91.2	17.4	$B3_{0.8}$	47.0	2.5	106.7	8.6
					$B3_{0.9}$	65.8	12.0	85.5	14.4
					$B3_{1.0}$	ND		66.5	14.4
$A4_{0.5}$	71.1	13.7	72.5	7.7	$B4_{0.5}$	73.6	6.8	58.1	9.0
$A4_{1.0}$	65.1	3.2	54.4	8.1	$B4_{1.0}$	57.8	2.2	61.3	16.5
$A5_0$	ND		66.7	15.7	$B5_0$	ND		55.8	10.4
$A5_{0.1}$	81.0	4.0	80.6	8.0	$B5_{0.2}$	66.7	3.0	62.9	10.4
$A5_{0.4}$	80.3	3.3	85.4	4.0	$B5_{0.8}$	60.1	3.6	94.0	10.6
$A5_{0.9}$	47.0	4.4	89.7	21.5	$B5_{1.0}$	47.6	5.1	107.0	5.6
$A5_{1.0}$	34.0	8.0	ND						
					$B6_{0.1}$	77.8	17.4	95.9	6.5
					$B6_{0.3}$	85.7	9.8	101.9	5.8
					$B6_{0.6}$	58.2	8.2	96.0	6.8
					$B6_{0.9}$	56.5	6.2	91.5	10.5
					$B6_{1.0}$	25.0	6.4	72.1	25.4

indicate dissimilarities in Cu binding among TLC bands within GPC fractions A3, B1, B5, and B6, whereas distinctions in Fe binding among TLC bands were observed in GPC fractions A1, B1, B2, B3, and B5. Multiple comparison tests demonstrated which TLC bands were distinct (Table 3-3). There, the bands are arranged according to descending order of percent yield.

In general, where differences in complexation are observed among TLC I bands (Table 3-3), those bands at lower R_f (R_f<0.4) tend to bind Cu more effectively. In contrast, Fe appears most effectively bound in bands of larger R_f, with the exception of A1 (Table 3-3). In fact, the orders are very nearly reversed in the birch series for Cu and Fe. The reversal may indicate a separation of Fe- and Cu-organic substances, implying the presence of ligands that preferentially bind one metal over another.

Binding of metals by TLC bands of aspen and birch soil extracts with the same R_f should be equivalent if the proposed ligands are the same. Any distinction in metal binding, indicated by differences in the percent yield of metals from the Chelex 100 analysis, implies the presence of dissimilar substances. The Mann-Whitney test was employed to ascertain any distinctions in Cu and Fe binding between TLC bands of aspen and birch samples possessing the same R_f value. The results are summarized

Table 3-3. Multiple comparison tests for average % yield of Cu and Fe among TLC I bands (1983 samples) within a forest type based on the of results of the Kruskal-Wallis test at the 5% level.

Cu

$A3_0$	$A5_{0.1}$	$B3_{0.4}$	$B5_{0.2}$	$B6_{0.1}$
$A3_{0.2}$	$A5_{0.4}$	$B3_{0.1}$	$B5_{0.8}$	$B6_{0.3}$
$A3_{0.9}$	$A5_{0.9}$	$B3_{0.9}$	$B5_{1.0}$	$B6_{0.6}$
	$A5_{1.0}$	$B3_0$		$B6_{0.9}$
		$B3_{0.8}$		$B6_{1.0}$

Fe

$A1_0$	$B1_{1.0}$	$B2_{0.3}$	$B3_{0.8}$	$B5_{1.0}$
$A1_{0.2}$	$B1_{0.2}$	$B2_{1.0}$	$B3_{0.9}$	$B5_{0.8}$
$A1_{1.0}$	$B1_{0.9}$	$B2_{0.9}$	$B3_{0.4}$	$B5_{0.2}$
	$B1_{0.1}$	$B2_{0.1}$	$B3_{0.1}$	$B5_0$
	$B1_0$	$B2_0$	$B3_{1.0}$	
			$B3_0$	

Vertical lines in the same column imply those means are not significantly different at the 5% level.
Overlapping vertical lines of two or more columns imply those means are not significantly different at the 5% level.
TLC band designations are:
A= aspen
B= birch
GPC fraction= 1-6
R_f= subscript 0-1.0

in Table 3-4. Among those fractions with the same R_f, five demonstrate significant differences in the percent copper recovered, and three in the percent recovery of iron. This result indicates the ability of substances from one vegetation type to bind metals more effectively than substances of the other vegetation type with comparable chromatographic characteristics.

Interestingly, it was found that the greatest quantity of copper and iron were in TLC I bands $A1_{0.2}$, $A2_{0.3}$, $A3_{0.4}$, $A4_{0.5}$, $A4_{1.0}$, and $B1_{0.2}$, $B2_{0.3}$, $B3_{0.4}$, $B4_{0.5}$, $B4_{1.0}$. This result, based on the total amount of iron and copper eluted from the TLC plates, agreed with previous work (Candler, 1985b). This implied that the substances contained in these TLC bands are the principal potential ligands for both Cu and Fe regardless of vegetation type. The chromatographic data, presented in the two previous chapters and the percent yield data presented above both suggest that soils from two forest types may contain substances with the same chromatographic characteristics but are dissimilar structurally. These dissimilar structures result in different metal binding characteristics as indicated by the percent yield data. These results further support the idea that vegetation-specific substances are produced and that substances from one forest type complex metals more readily than another.

Table 3-4. Significant differences, as determined by the Mann-Whitney test, in Cu and Fe binding between birch and aspen TLC I bands with the same R_f within a GPC fraction of equivalent K_d. 1983 samples.

TLC Band	Significant Difference Cu	Fe
$A1_0$ vs $B1_0$	-	-
$A1_{0.2}$ vs $B1_{0.2}$	+	-
$A2_0$ vs $B2_0$	-	-
$A2_{0.1}$ vs $B2_{0.1}$	-	-
$A2_{0.3}$ vs $B2_{0.3}$	+	-
$A2_{0.9}$ vs $B2_{0.9}$	+	-
$A2_{1.0}$ vs $B2_{1.0}$	NA	+
$A3_0$ vs $B3_0$	+	+
$A3_{0.4}$ vs $B3_{0.4}$	+	+
$A3_{0.9}$ vs $B3_{0.9}$	-	-
$A4_{0.5}$ vs $B4_{0.5}$	-	-
$A4_{1.0}$ vs $B4_{1.0}$	-	-
$A5_{0.1}$ vs $B6_{0.1}$	-	-
$A5_{0.9}$ vs $B6_{0.9}$	-	-

+= significant difference at 5% level
-= no significant difference at the 5% level
NA= not available
A= aspen
B= birch
GPC fraction= 1-6
R_f= subscript 0-1.0

1984 Samples--TLC I

For the 1983 samples, it was noted, that in some instances the quantity of metal eluted from the plates exceeded the quantity of metal applied to the plates. Excess metal was thought to be background extracted from the plates, external contamination, or both. If background metals were derived from the plates (assuming consistent metal content), similar patterns in excesses would be expected in the mass balance (i.e. excess Fe in A2, A4, B3, B4; excess Cu in A5), since TLC characteristics were identical to those of the 1983 samples. It can be seen from Table 3-5 that excess Fe is found in A3, A4, A5, and B1, whereas, excess Cu is found in virtually all of the birch-derived material. Microgram percent excess of copper ranged from 30 to 60 percent while excess iron ranged from 17 to 60 percent. The phenomenon is not clearly understood. It may be due to yearly variations in production of the amount of organic substances that may extract metal from the TLC plates or be due to contamination from sample handling. However, the utmost care was observed to avoid contamination in all stages of this study.

Copper and iron appeared to be concentrated in the same TLC fractions observed by Candler (1985b) which include $B2_{0.3}$, $B3_{0.4}$, $B4_{0.5}$, $A2_{0.3}$, $A3_{0.4}$, and $A4_{0.5}$. Table (3-6) summarizes percent yield of Fe and Cu for each

Table 3-5. Mass balance for Cu and Fe (in µg) for the TLC I procedure (1984 samples).

GPC Fraction	Plate #	Cu on	Cu off	Fe on	Fe off
A1	1	4.4	4.5	33.8	*37.4*
	2	4.4	4.8	33.8	29.2
A2	1	20.2	NA	145.7	132.7
	2	15.5	14.4	112.1	87.7
A3	1	21.8	12.8	237.0	*309.2*
	2	21.8	12.4	237.0	*295.5*
A4	1	19.0	12.4	48.0	*78.7*
	2	19.0	12.0	48.0	*67.2*
A5	1	12.1	12.8	17.1	*28.2*
	2	11.2	*14.8*	15.7	*27.6*
B1	1	9.4	8.8	156.4	*202.2*
	2	9.4	*11.1*	156.4	*183.9*
B2	1	8.0	4.7	160.2	15.5
	2	1.7	*2.4*	156.5	17.3
B3	1	14.4	*19.1*	266.1	267.4
	2	5.0	*8.6*	93.1	90.2
B4	1	9.9	*15.3*	42.3	41.1
	2	9.9	*15.6*	42.3	44.7
B5	1	3.0	3.4	11.2	11.4
	2	3.0	*4.0*	11.5	13.7
B6	1	3.6	*4.8*	28.9	16.9
	2	3.6	*5.5*	28.9	20.0

Table 3-6. Average % yield, ± standard error, of Fe and Cu for each 1984 TLC I band (two replicate plates with two determinations per TLC band, n=4).

Fraction	%yield Cu	SE	Fe	SE	Fraction	%yield Cu	SE	Fe	SE
$A1_0$	46.2	±4.8	89.2	±5.7	$B1_0$	85.9	±2.3	88.1	±5.4
$A1_{0.2}$	64.5	±2.1	75.4	±8.6	$B1_{0.1}$	88.5	±2.7	88.9	±5.7
$A1_{1.0}$	39.0	±7.5	64.6	±6.5	$B1_{0.2}$	85.0	±1.1	100.3	±4.3
					$B1_{0.9}$	90.9	±4.6	79.3	±3.6
					$B1_{1.0}$	ND		83.1	±17.0
$A2_0$	59.8	±2.7	84.7	±16.8	$B2_0$	92.3	±3.4	83.2	±0.6
$A2_{0.1}$	73.2	±0.9	84.2	±4.1	$B2_{0.1}$	79.4	±1.1	78.6	±0.8
$A2_{0.3}$	77.2	±3.3	85.0	±2.8	$B2_{0.3}$	93.8	±2.2	98.3	±2.5
$A2_{0.9}$	80.4	±20.8	58.1	±3.7	$B2_{0.9}$	90.1	±1.9	85.5	±6.2
$A2_{1.0}$	ND		91.2	±11.2	$B2_{1.0}$	ND		68.1	±17.7
$A3_0$	55.2	±7.1	100.0	±5.5	$B3_0$	72.7	±7.3	106.2	±8.8
$A3_{0.4}$	81.0	±1.8	94.8	±2.2	$B3_{0.1}$	70.0	±10.9	85.2	±20.0
$A3_{0.9}$	56.7	±1.3	66.4	±1.7	$B3_{0.4}$	65.6	±4.4	69.2	±5.6
$A3_{1.0}$	63.9	±2.5	49.2	±5.0	$B3_{0.8}$	78.9	±3.7	80.0	±1.4
					$B3_{0.9}$	85.9	±2.3	ND	
					$B3_{1.0}$	ND		ND	
$A4_{0.5}$	65.1	±6.2	96.7	±5.9	$B4_{0.5}$	74.6	±2.1	92.5	±3.1
$A4_{1.0}$	68.6	±2.4	129.0	±4.5	$B4_{1.0}$	54.9	±4.7	97.1	±1.9
$A5_0$	70.1	±11.1	44.6	±4.2	$B5_0$	88.1	±13.7	79.0	±12.6
$A5_{0.1}$	70.4	±3.2	84.8	±19.9	$B5_{0.1}$	86.7	±4.6	106.5	±5.9
$A5_{0.4}$	89.7	±4.6	68.8	±9.7	$B5_{0.8}$	90.3	±1.2	99.8	±4.9
$A5_{0.9}$	34.5	±1.1	91.5	±0.7	$B5_{1.0}$	54.8	±1.2	98.7	±0.8
$A5_{1.0}$	24.9	±4.3	ND						
					$B6_{0.1}$	77.7	±7.9	74.4	±9.1
					$B6_{0.3}$	75.4	±7.1	75.8	±7.1
					$B6_{0.6}$	60.3	±1.3	56.6	±5.8
					$B6_{0.9}$	311.0	±4.2	52.8	±17.7
					$B6_{1.0}$	ND		59.3	±3.2

TLC fraction of aspen and birch soil extracts. The possible total number of TLC bands that vigorously bind Cu in aspen soil-generated material was 5 and in birch there were 16. Those vigorously binding Fe numbered 12 in aspen and 21 in birch. The total number of TLC bands strongly binding Fe was very similar compared to the 1983 data aspen (15) and birch (17), whereas the number vigorously binding Cu were nearly doubled in aspen (1983: 9-10) and halved in birch (1983: 7) samples. The observed differences in the number of TLC bands that bound Cu and Fe strongly for the two years indicated yearly variations in ligand production and structure.

Variances for these data were heteroscedastic and required the use of non-parametric statistical tests. The average percent yield of copper over all TLC bands, for both forest types, indicated that copper was not strongly bound. Results of the Mann-Whitney test showed that Cu was more effectively bound by components in the birch soil extract than those in the aspen soil extract. This result is in contrast to that of 1983. The average percent yield of Cu for 1983 was: aspen 73.6 percent and birch 64.9 percent, whereas, the average percent yield for 1984 was: aspen 61.5 percent and birch 76.7 percent. Further, these results indicated yearly variations in Cu binding between the forest types. On the other hand, iron was bound equally strongly by components in the aspen and

birch extracts for both years. The average percent yield of iron for 1983 was: aspen 78.9 percent and birch 80.2 percent. The average percent yield for 1984 was: aspen 83.7 percent and birch 83.5 percent.

Results of the Kruskal-Wallis test (Table 3-7) for both vegetation types indicated that significant differences existed among TLC fractions binding Cu within a given GPC fraction. The 1984 aspen results were similar to those for aspen in 1983. The 1984 sample results for copper binding by the birch extracts were virtually the opposite from the 1983 results. Binding of iron appeared to be uniform among TLC bands within a given birch GPC fraction from the 1984 samples. These results were quite different from those for 1983. TLC bands within aspen GPC fractions A2, A3, and A5 appeared to bind Fe uniformly for both years. Significant differences in bound Fe among TLC bands within aspen GPC fraction A1 were observed for 1983 whereas significant differences were observed within A4 for 1984. These data may indicate yearly differences in metal binding and may be worthy of further research to determine the yearly variations.

Multiple comparisons revealed the groupings of bands which bound Cu most effectively. These groupings were fractions generally with the lowest R_f values, a result consistent with the 1983 data (Table 3-8).

Table 3-7. Summary of results for the Kruskal-Wallis test for significant differences in Cu and Fe binding among TLC bands within a GPC fraction for both years at the 5% level.

GPC Fraction	Cu significant difference 1984	Cu significant difference 1983	Fe significant difference 1984	Fe significant difference 1983
A1	+	-	-	+
A2	-	-	-	-
A3	+	+	-	-
A4	-	-	+	-
A5	+	+	-	-
B1	-	-	-	+
B2	+	-	-	+
B3	-	+	-	+
B4	+	-	-	-
B5	-	+	-	+
B6	+	+	-	-

+= significant difference in metal binding
-= no significant difference in metal binding

Table 3-8. Multiple comparison tests for average Cu % yield among TLC I bands (1984 samples) within a forest type based on the results of the Kruskal-Wallis test at the 5% significance level.

$A1_{0.2}$	$A3_{0.4}$	$A5_{0.4}$	$B2_{0.3}$	$B6_{0.1}$
$A1_{0}$	$A3_{1.0}$	$A5_{0}$	$B2_{0.9}$	$B6_{0.3}$
$A1_{1.0}$	$A3_{0}$	$A5_{0.1}$	$B2_{0}$	$B6_{0.6}$
	$A3_{0.9}$	$A5_{0.9}$	$B2_{0.1}$	$B6_{0.9}$
		$A5_{1.0}$		

Vertical lines in the same column implies those means are not significantly different at the 5% level.
Overlapping vertical lines of two or more columns implies those means are not significantly different at the 5% level.
TLC band designations are:
A= aspen
B= birch
GPC fraction= 1-6
R_f= subscript 0-1.0

Metal binding distinctions existed between substances derived from aspen and birch material of equivalent R_f values within a comparable GPC fraction (Table 3-9). Thin-layer chromatographic bands with the same R_f may contain substances that are structurally the same or similar. If substances are structurally the same then equivalent metal binding characteristics, expressed as percent yield, would be expected. Compounds belonging to the same compound class are structurally similar. Under the conditions that the TLC was carried out in this study, these substances may exhibit the same TLC characteristics, as reflected by R_f values. Structures in this instance may be sufficiently different to provide distinct metal binding characteristics. The data in Table (3-9) suggest that ligands of sufficiently dissimilar structure are produced by different vegetation types.

Several TLC bands consistently exhibited significant differences in Cu binding between aspen and birch soil extracts over the two-year period. These bands are: $A1_{0.2}$,$B1_{0.2}$; $A2_{0.3}$, $B2_{0.3}$; and $A3_{0.4}$, $B3_{0.4}$. However, in the 1983 samples, $A1_{0.2}$ and $A2_{0.3}$ bound Cu strongly and more effectively than $B1_{0.2}$ and $B2_{0.3}$, whereas $B3_{0.4}$ bound Cu strongly and more effectively than $A3_{0.4}$. The same TLC bands obtained from the 1984 samples were reversed, i.e. $B1_{0.2} > A1_{0.2}$, $B2_{0.3} > A2_{0.3}$, and $A3_{0.4} > B3_{0.4}$. Yearly variations in ligand production may be responsible

Table 3-9. Significant differences, as determined by the Mann-Whitney test, in Cu and Fe binding between birch and aspen TLC I bands with the same R_f within a GPC fraction with equivalent K_d. 1984 samples.

TLC band	Significant Difference Cu	Fe
$A1_0$ vs $B1_0$	+	-
$A1_{0.2}$ vs $B1_{0.2}$	+	-
$A1_{1.0}$ vs $B1_{1.0}$	NA	-
$A2_0$ vs $B2_0$	+	-
$A2_{0.1}$ vs $B2_{0.1}$	+	-
$A2_{0.3}$ vs $B2_{0.3}$	+	+
$A2_{0.9}$ vs $B2_{0.9}$	-	+
$A2_{1.0}$ vs $B2_{1.0}$	NA	-
$A3_0$ vs $B3_0$	-	-
$A3_{0.4}$ vs $B3_{0.4}$	+	+
$A3_{0.9}$ vs $B3_{0.9}$	-	NA
$A4_{0.5}$ vs $B4_{0.5}$	-	-
$A4_{1.0}$ vs $B4_{1.0}$	-	+
$A5_{0.1}$ vs $B6_{0.1}$	-	-
$A5_{0.9}$ vs $B6_{0.9}$	-	-

+= significant difference at 5% level
-= no significant difference at 5% level
NA= not available
TLC band designations are:
A= aspen
B= birch
GPC fraction= 1-6
R_f= subscript 0-1.0

for this phenomenon. However, the explanation for these differences is still unclear. Over a two-year period, iron was bound more effectively by $A3_{0.4}$ than $B3_{0.4}$, and $B2_{0.3}$ than $A2_{0.3}$.

By combining the results from the 1983 and 1984 samples a grand mean was obtained for the percent yield of each TLC band within a GPC fraction. Comparison of copper binding between TLC bands of equivalent R_f within GPC fractions of equivalent K_d obtained from aspen and birch soil extracts revealed that there were no significant differences in the amount of Cu recovered except in "minor" TLC fractions which are those fractions containing relatively small quantities of metal (Table 3-10). TLC bands with R_f = 0, and R_f = 0.6-1.0 usually possessed the smallest metal content. This was not the case with the binding of iron: significant differences appeared between both minor and principal bands of equivalent R_f values. Two bands, most notably $GPC2_{0.3}$ and $GPC3_{0.4}$ which contain the largest quantities of iron, demonstrated distinct iron binding characteristics consistently over two years. The band $B2_{0.3}$ bound iron more effectively than $A2_{0.3}$ whereas $A3_{0.4}$ was binding iron more effectively than $B3_{0.4}$. This result suggested that substances in these bands may be vegetation-specific, and therefore distinct chemically although the chromatographic data indicated the same or similar substances were present.

Table 3-10. Average for both years (1983,1984) of % yield for Cu and Fe showing significant differences, as determined by the Mann-Whitney test, in Cu and Fe binding between aspen and birch TLC I bands with the same R_f within a GPC fraction of equivalent K_d. (n=12 for all except birch GPC fraction 6, n=10)

GPC fraction	R_f	Cu	Fe	Average % yield ±standard error Cu Aspen	Cu Birch	Fe Aspen	Fe Birch
1	0	+	-	64.1 ± 5.4	81.7 ± 2.4	85.8 ± 6.4	71.8 ± 6.2
	0.2	-	-	78.1 ± 4.2	78.6 ± 2.8	77.7 ± 4.5	77.8 ± 3.9
	1.0	-	+	59.3 ± 8.8	NA	54.2 ± 6.9	103.1 ±10.0
2	0	-	-	83.5 ± 5.0	71.9 ± 5.6	76.9 ± 6.7	59.8 ± 4.7
	0.1	-	+	80.6 ± 5.4	66.5 ± 6.8	90.2 ± 2.1	68.1 ± 8.5
	0.3	-	+	89.2 ± 4.6	82.9 ± 3.4	84.8 ± 1.8	93.9 ± 2.1
	0.9	-	-	89.9 ± 9.0	62.7 ±10.4	78.4 ± 9.6	96.1 ± 8.5
3	0	-	-	81.7 ± 3.5	64.8 ± 6.4	86.3 ± 4.7	53.9 ±11.1
	0.4	-	+	73.1 ± 3.5	78.7 ± 3.1	95.2 ± 1.4	76.2 ± 4.4
	0.9	-	-	61.7 ± 8.2	71.4 ± 7.8	80.0 ±10.9	NA
4	0.5	-	-	69.1 ± 3.2	73.9 ± 4.2	80.5 ± 6.1	73.2 ±11.4
	1.0	+	-	66.4 ± 2.2	56.6 ± 2.1	84.2 ±12.5	63.5 ± 8.1
A5(B6)	0.1	-	-	77.1 ± 3.0	77.8 ± 9.9	82.0 ± 4.4	87.3 ± 5.8
A5(B6)	0.9	+	-	42.9 ± 3.2	46.4 ± 5.4	90.2 ±14.7	74.3 ±10.7

NA= not available

Ion Exchange Chromatography--TLC II

It was stated earlier that $A2_{0.3}$, $A3_{0.4}$, $A4_{0.5}$, $B2_{0.3}$, $B3_{0.4}$, and $B4_{0.5}$ contained the largest quantities of copper and iron. Therefore these TLC bands were chosen for further chromatographic studies because these bands probably contained the principal ligands. Unfortunately the quantity of material representing $A2_{0.3}$ was insufficient to carry out the percent yield studies for comparison with those of $B2_{0.3}$. Therefore, only the results for $B3_{0.4}$ versus $A3_{0.4}$ and $A4_{0.5}$ versus $B4_{0.5}$ are discussed.

A total of 20 to 50 microgram percent (micrograms of metal recovered from the TLC plate/micrograms of metal applied to the TLC plate) excess copper appeared to be eluted from the plates in each TLC II procedure. In some instances iron exhibited up to 25 percent excess eluted from the TLC plates. This excess suggested that additional metal was extracted from the TLC sorbent but owing to the amount of plate handling during this fractionation procedure, external contamination may be a more reasonable explanation for this phenomenon. For this reason a discussion of metal distribution among the TLC II bands may appear dubious, particularly in the case of copper. However, the maximum microgram percent of copper was not associated with the same band in all cases. This fact suggests dissimilar

Cu-organic associations even though organic substances applied to the plates may have extracted metals from the plates. The greatest iron content (in microgram percent) was always associated with the last band (band 6). It is reasonable to suggest that the organic substances contained in band 6 exhibit a greater affinity for iron than copper, whereas the reverse is true for bands 1 to 3. This result indicates a moderate separation of supposed Fe- and Cu-organic chelates. Microgram percent of iron and copper is tabulated in Table (3-11).

Table (3-12) summarizes the percent yields of copper and iron from the Chelex column. These data indicate that substances in most of these TLC bands strongly complex iron and copper. Stevenson (1985), in a review of trace metal complexation by humic substances, suggested that the ability to form complexes may be attributed to the preponderance of oxygen-containing functional groups. These functional groups include carboxylic acid (-COOH), various carbonyl (-C=O), phenolic hydroxyl, and enolic groups. Nitrogen containing groups, such as amino groups, may also participate in complexation of metals. Evidence indicates chelation of trace metals, such as iron and copper, is primarily achieved by the carboxylate anion ($-COO^-$). Most of the copper appeared to be associated with bands exhibiting strong fluorescence (TLC II bands

Table 3-11. Average microgram percent, ± standard error, of Cu and Fe in each TLC II band observed in TLC I bands $A3_{0.4}$, $B3_{0.4}$, $A4_{0.5}$, and $B4_{0.5}$ (Two replicate plates with two metal determinations per TLC band).

TLC II Band	$A3_{0.4}$ Fe	$A3_{0.4}$ Cu	$B3_{0.4}$ Fe	$B3_{0.4}$ Cu	$A4_{0.5}$ Fe	$A4_{0.5}$ Cu	$B4_{0.5}$ Fe	$B4_{0.5}$ Cu
1	1.8±0.6	26.0±3.7	1.5±0.1	15.4±0.8	13.6±0.7	30.2±3.1	8.0±0.3	60.3±6.4
2	5.7±0.4	31.5±3.0	10.1±1.4	30.4±1.6	22.9±1.3	16.9±1.9	9.5±0.6	20.4±1.1
3	4.8±1.2	16.1±1.2	12.6±1.2	30.1±2.3	6.1±0.1	14.0±1.8	10.1±0.8	13.9±0.2
4	10.6±0.3	16.9±0.3	17.5±1.4	24.1±2.4	7.4±0.8	11.4±0.2	12.0±0.9	13.9±0.2
5	11.0±0.4	12.2±0.6	17.2±0.6	17.1±0.4	12.9±0.8	15.2±0.9	9.7±0.6	6.7±0.6
6	47.9±6.7	23.8±1.5	44.2±3.3	18.3±0.5	26.1±1.0	20.5±0.6	58.1±7.8	20.6±2.5

Table 3-12. Average percent yield, ± standard error, of Fe and Cu for each TLC II band observed in TLC bands $A3_{0.4}$, $B3_{0.4}$, $A4_{0.5}$, and $B4_{0.5}$.

Band	$A3_{0.4}$ Fe	$A3_{0.4}$ Cu	$B3_{0.4}$ Fe	$B3_{0.4}$ Cu	$A4_{0.5}$ Fe	$A4_{0.5}$ Cu	$B4_{0.5}$ Fe	$B4_{0.5}$ Cu
1	72.3±7.2	62.8±8.7	100+	64.2±17.2	72.3 ±6.0	82.2±2.5	83.4 ±1.7	81.9±3.7
2	83.1±5.1	81.7±3.9	68.±110.3	65.5±11.5	61.1±12.0	72.3±2.7	78.7 ±2.9	81.5±2.1
3	71.7±3.5	78.8±2.8	76.6± 0.8	74.2±11.2	73.3±10.2	66.8±1.7	64.6 ±1.9	78.2±1.8
4	82.3±2.4	84.1±3.2	82.7± 2.8	79.5 ±4.9	84.3 ±2.6	84.5±3.6	61.7 ±2.2	83.0±2.4
5	75.9±1.6	78.5±3.0	93.7± 2.2	82.1±12.4	81.5 ±3.8	84.2±0.7	46.2 ±2.5	79.2±2.4
6	89.8±4.0	87.8±2.3	93.0± 1.8	88.3 ±1.4	102.1 ±0.5	88.0±1.5	67.8±13.9	73.7±4.2

1-3, see chapter 2). Fluorescence in organic substances usually indicates the presence of conjugated double bonds. Infrared spectra of $A3_{0.4}$1-3, $B3_{0.4}$1-3, $A4_{0.5}$1-3, and $B4_{0.5}$1-3 (see chapter 2) strongly indicated the presence of undissociated -COOH, $-COO^-$, and possibly other -C=O functional groups capable of chelating transition metals. The band contours in these spectra indicated that ligands bound with most of the copper were structurally different than those bound to most of the iron.

Most of the iron is generally associated with the non-fluorescing bands (bands 4-6). This result suggests little or no conjugation of double bonds is present in these materials. The infrared spectra of these bands indicated a preponderance of $-COO^-$ with very little undissociated -COOH present. In light of these results and the fact that iron is generally strongly complexed in bands 4-6, the principal group complexing iron is probably $-COO^-$.

Results of the Kruskal-Wallis test demonstrated that binding of iron within aspen was the same among bands whereas significant differences in percent yield were observed among bands in $B3_{0.4}$1-6 and $B4_{0.5}$1-6. The opposite was true for copper complexation in that significant dissimilarities were observed among bands originating from aspen extracts only. Multiple comparison tests (Table 3-13) revealed the distinctions in

copper complexation within $A3_{0.4}$, $A4_{0.5}$, and iron complexation within $B3_{0.4}$, $B4_{0.5}$. Substances contained in $A3_{0.4}4$, $A3_{0.4}6$, $A4_{0.5}4$, and $A4_{0.5}6$ appeared to most strongly complex copper. As noted earlier, these bands are non-flourescing and the principal chelating groups are probably $-COO^-$. Iron is strongly complexed by $B3_{0.4}1$, $B3_{0.4}6$, $B3_{0.4}5$, and $B3_{0.4}4$. These results suggest, for the reasons stated previously, that, with the exception of $B3_{0.4}1$, $-COO^-$ were the principal functional groups chelating iron. Bands $B4_{0.5}1$ and $B4_{0.5}2$, on the other hand, strongly complex iron. These two results must be viewed with suspicion because the iron concentration in these two TLC bands was near the detection limits of the instrument. These bands show strong fluorescence and indicated the presence of conjugated double bonds. These results indicate that vegetation-type-specific organic substances preferentially complex one metal over another.

Comparisons of $B3_{0.4}1$-6 versus $A3_{0.4}1$-6 and $A4_{0.5}1$-6 versus $B4_{0.5}1$-6 using Kruskal-Wallis test further substantiate dissimilarities in copper and iron binding between substances originating from different vegetation types. Multiple comparisons reveal which bands are different (Table 3-14). There are clear distinctions in iron and copper complexation between aspen and birch materials. Bands $B3_{0.4}1$ and $A3_{0.4}1$ strongly complex iron; however, band $B3_{0.4}1$ binds iron most effectively.

Table 3-13. Multiple comparisons tests for average % yield of Cu and Fe among TLC II bands within a forest type based on the results of the Kruskal-Wallis test at the 5% significance level.

Fe				Cu			
$B3_{0.4}1$	$B4_{0.5}1$	$A3_{0.4}4$	$A4_{0.5}6$
$B3_{0.4}6$	$B4_{0.5}2$	$A3_{0.4}6$	$A4_{0.5}4$
$B3_{0.4}5$	$B4_{0.5}3$	$A3_{0.4}2$	$A4_{0.5}5$
$B3_{0.4}4$	$B4_{0.5}6$	$A3_{0.4}3$	$A4_{0.5}1$
$B3_{0.4}2$	$B4_{0.5}4$	$A3_{0.4}5$	$A4_{0.5}2$
$B3_{0.4}3$	$B4_{0.5}5$	$A3_{0.4}1$	$A4_{0.5}3$

Vertical lines in the same column imply those means are not significantly different at the 5% level.
Overlapping vertical lines of two or more columns imply those means are not significantly different at the 5% level.
TLC band designations are:
A3= aspen GPC fraction three
A4= aspen GPC fraction four
B3= birch GPC fraction three
B4= birch GPC fraction four
R_f= subscript
TLC II band=1-6

Table 3-14. Multiple comparisons tests for average % yield of Cu and Fe among TLC II bands between the two forest types based on the results of the Kruskal-Wallis test.

$A3_{0.4}$ 1-6 vs $B3_{0.4}$ 1-6	$A4_{0.5}$ 1-6 vs $B4_{0.5}$ 1-6
Fe	
$B3_{0.4}1$ |	$A4_{0.5}6$ |
$A3_{0.4}6$ |	$A4_{0.5}4$ | |
$B3_{0.4}5$ |	$B4_{0.5}1$ | |
$B3_{0.4}6$ |	$A4_{0.5}5$ |
$A3_{0.4}2$ |	$B4_{0.5}2$ |
$B3_{0.4}4$ | |	$A4_{0.5}3$ | |
$A3_{0.4}4$ | |	$A4_{0.5}1$ | |
$B3_{0.4}2$ | |	$B4_{0.5}6$ | |
$A3_{0.4}1$ |	$A4_{0.5}2$ | |
$A3_{0.4}3$ |	$B4_{0.5}3$ | |
$B3_{0.4}3$ |	$B4_{0.5}4$ | |
$A3_{0.4}5$ |	$B4_{0.5}5$ |
Cu	
$A3_{0.4}4$ |	$A4_{0.5}6$ |
$A3_{0.4}6$ |	$A4_{0.5}5$ |
$B3_{0.4}6$ |	$A4_{0.5}4$ | |
$B3_{0.4}3$ | |	$B4_{0.5}4$ | |
$B3_{0.4}1$ |	$A4_{0.5}1$ |
$B3_{0.4}5$ |	$B4_{0.5}1$ |
$A3_{0.4}2$ |	$B4_{0.5}2$ | |
$B3_{0.4}4$ |	$B4_{0.5}5$ | |
$B3_{0.4}2$ |	$B4_{0.5}3$ | |
$A3_{0.4}3$ |	$B4_{0.5}6$ | |
$A3_{0.4}5$ | |	$A4_{0.5}2$ |
$A3_{0.4}1$ |	$A4_{0.5}3$ |

A3= aspen GPC fraction three
A4= aspen GPC fraction four
B3= birch GPC fraction three
B4= birch GPC fraction four
R_f= subscript
TLC II band =1-6

One must consider this result in the context that the iron content in these two bands is very low, and the iron analysis is approaching the detection limits of the instrument. Strong complexation of iron is observed for bands $B3_{0.4}5$, $A4_{0.5}4$, and $A4_{0.5}$. Corresponding bands $A3_{0.4}5$, $B4_{0.5}4$, and $B4_{0.5}5$ showed significant differences in the binding of iron. Strong copper complexation was observed in bands $A3_{0.4}1$, $B4_{0.5}2$, $B4_{0.5}3$, and $A4_{0.5}6$. These bands exhibited significant differences in copper binding from the corresponding bands: $B3_{0.4}1$, $A4_{0.5}2$, $A4_{0.5}3$, and $B4_{0.5}6$. These results clearly demonstrate differential metal complexation by substances from different vegetation types. These data are intriguing in that they suggest better, and more definitive separations may be feasible.

Conclusions

1. No significant difference in overall iron complexation occurs between aspen and birch soil extracts for the two years 1983, and 1984. Strong overall iron binding is observed in both forest types. Overall complexation of copper exhibited significant differences between the two forest types for both years. In 1983, copper complexation was greater in the aspen material than in the birch material. The opposite condition existed for the 1984 samples. This result indicates yearly

variations in the production of organic materials involved with copper complexation.

2. Generally, copper appears to be most effectively bound by TLC I bands with R_f<0.4, whereas iron is usually most effectively bound by TLC I bands with R_f>0.3 regardless of vegetation type. This difference suggests partial separation of iron- and copper-complexes.
3. Significant differences in metal complexation by substances contained in TLC I bands are observed within and between the two forest types. This result indicates vegetation-type specific complexing agents are produced.
4. Further chromatographic separations, evident in the ion exchange data from the TLC II procedure, clearly indicates the presence of dissimilar metal complexes within and between aspen and birch soil extracts.
5. The principal functional groups chelating iron probably are $-COO^-$.
6. The data presented in this thesis strongly indicate that many components found in the aspen extracts are chemically distinct from those found in the birch extracts. Furthermore, these data strongly support the idea vegetation-type-specific suites of mobile, water soluble chelates exist.

Summary and Suggestions for Future Research

Synthetic chelates are well known and are often used to fertilize agricutural soils depleted in micronutrients such as iron. Iron in solution is easily oxidized, by air, to form insoluble iron oxide. Iron in this form is immobile and unavailable for plant uptake which is necessary for healthy plant growth. Synthetic chelating agents can maintain a wide variety of micro-nutrients, such as iron, in a soluble form (Lindsay, 1979). Natural chelates are present in most soils. Ligand structure in these chelates is unknown precluding comprehension of the reactions in which they participate. Lindsay (1979) states:

> "... investigations of complexing by soil organic matter that leads to the identification of specific complexes or chelates and the thermodynamic constants controlling their reactions are to be encouraged."

I believe that the study of metal-chelates derived from the environment is principally a chromatographic problem, and that efforts to elucidate structure of the ligands or binding mechanisms should be preceded by obtaining chromatographically pure substances.

The results from gel-permeation chromatography showed consistent separations for aspen and birch soil extracts over the two-year study period. The elution curves obtained from this technique indicated that the

aspen soil extracts differed in composition from the birch soil extracts. Further separations of the components comprising the gel-permeation fractions were effected by the thin-layer chromatographic procedures used. The results from thin-layer chromatography indicated differences in chemical composition within and between fractionated substances derived from aspen and birch soil extracts. Significant distinctions in copper and iron complexing among components within and between vegetation-type extracts were observed in the data from ion exchange data. The infrared spectra did not provide detailed structural information about the organic ligands.

Complexation of iron probably occurred through bonding with carboxylate anions, whereas, copper complexation may have occurred via bonding with carboxylate anions and some other oxygen containing functional group. Furthermore, partial separations of copper and iron complexes in the extracts were indicated. The results of this study strongly support the hypothesis that rate differences of element cycling observed between aspen and birch forest soils are, in part, attributable to forest-type-specific suites of mobile, water-soluble chelates.

Another important result of this work is the advance of an inexpensive method for detailed investigation of naturally generated chelates extracted from soil. The method is still qualitative and needs refinement. Thin-layer chromatographic procedures can be improved. For example, the sorbent must be free of the metal or metals of interest. An attempt can be made to make this stage of the separations quantitative. Theoretically, any separation achieved by thin-layer chromatography may be achieved by high-performance liquid chromatography. This technique would be extremely advantageous because the time required for analysis could be diminished at least ten fold. Some investigations concerning copper-chelates originating from the marine environment have successfully employed C^{18} reverse phase liquid chromatography (Donat et al., 1985; Hanson, 1981; Mantoura et al., 1978; Mills et al., 1981).

After being refined, the thin-layer chromatography procedure should be applied to similar studies of several contiguous aspen and birch forest soils to ascertain the generality of the central hypothesis stated earlier. The methods outlined in this thesis could be applied to the metal binding by organic components throughout an entire soil profile as well as to a wide variety of vegetation types. The results should provide improved understanding

of micronutrient availability and cycling, the role of chelates in soil formation, and the possible uses of chelation for preferential extraction of metals from ores.

I believe a discussion of the ecological implications of this work would be premature. Experiments, using the methods described, should be designed to determine seasonal variations in metal complexation. Furthermore, throughfall and stemflow must be studied using these methods to determine the contributions of metal complexation by organic substances derived from these sources.

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