

美國 北部 활엽수림 litter로부터 發生되는 有機酸의 탄소-13 NMR 分析*1

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Carbon-13 NMR Analysis of Organic Carbons from a Northern Hardwood Litter and Leachates, Central Maine, U.S.A. *1

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要 約

나무로부터 발생되는 유기물질이 자연상태에서 어떻게 변화되는가를 탄소-13 핵자기 공명 스펙트럼에 의하여 분석하였다. Litter를 구성하는 주요탄소의 형태는 지방족 탄화수소, 탄수화물, 그리고 방향족 탄소들이었으며, 메톡실, 카르복실과 카르보닐형 탄소들은 부후의 정도가 커짐에 따라 리그닌의 농도가 litter 중에서 증가하므로 점차 증가한다. Hydrophobic과 hydrophilic형 유기탄소가 litter의 자연 여과액(leachates)에 많이 존재하며, 그들의 탄소의 구조는 산성, 염기성 그리고 중성으로 분류되었을 때 서로 특이한 탄소의 형태를 갖는다.

Keywords : Litter, leachates, carbon-13 NMR

1. INTRODUCTION

Litter and litter leachates play an important role in the chemistry of terrestrial and aquatic ecosystem.¹⁾ Extensive researches have been conducted on the inorganic properties of litter and leachates. Recently the role of leachates has an emphasis biologically and chemically in the forest ecosystem.²⁾ In order to investigate the biological and chemical role in the ecosystem, the chemical characteristic of litter and leachates should be evaluated.

High molecular weight compounds such as lignin³⁾, humic acid⁴⁾, fulvic acid⁵⁾ and polymeric nucleic acids present in natural waters

are considered as the precursors of chlorinated compounds. These materials cause problems in public water supplies, and they may combine with and concentrate toxic metals, and react with chlorine to produce chloroform and other halogenated organics such as THM^{4,6)}. Understanding of the chlorination mechanism has been slow because the origin, nature, fate and chemical structure of humic substances which are highly decomposed organic compounds derived from plant and microbial synthesis is still not fully understood.

Some early attempts were focused on the use of proton NMR spectroscopy to investigate the different types of proton in litter, but

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the spectra were not reliable for aromatic and olefinic protons because of solvent effects and relaxing effects of spins of unpaired electrons.⁷⁾

In stead, carbon-13 NMR spectroscopic techniques have proven to be valuable tools for the characterization of litter.^{8,9)} This is especially true for humic substances which are very decomposed high molecular weight organic substances since very small amount of sample is required, and detailed information can be obtained about all of the important carbon-containing functional groups.^{10,11)} However, early attempts to obtain usable spectra by solid carbon-13 NMR provided little information because of technical problems. Development of cross polarization (CP) of carbons by protons matched with magic angle spinning (MAS), finally made it possible to achieve 13C resolution approaching that of solution NMR.^{11,12,13)}

The purposes of this study were to better understand the chemical composition of litter and litter leachates, and to investigate the changes in chemical composition of organic carbon in litter leachates with seasonal variation by using a new solid state carbon-13 NMR spectroscopy.

2. MATERIALS & METHODS

2.1 Study Site

The study site is located in the Bear Brooks Watershed in Maine. Vegetation is composed mainly of northern hardwood species, including American Beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula allegheniensis* Britton), striped maple (*Acer pennsylvanicum* L.) and sugar maple (*Acer saccharum* Marsh.), with some inclusions of red spruce (*Picea rubens* Sarg.). Site elevation is 1015 m with an average annual precipitation of 131cm.²⁾

2.2 Samples

Slightly decomposed litter was collected, and kept in plastic bag, and dried at room temperature in the laboratory. Samples were

ground in a Wiley mill until they passed through a 100 mesh sieve, and refrigerated.

2.3 Litter Leachate Collection and Fractionation

Litter leachates were collected by using lysimeters (90cm wide, 120cm long, 12cm deep). Beginning in June 1992, 16L samples were collected monthly until October 1992 and filtered through 0.7 μ m fiber glass (GF/F) and stored at 3°C. Dissolved organic carbon was measured by CO₂ IR absorbance of UV-persulfate oxidation products of DOC (Dohrmann DC 80 Carbon Analyzer, Santa Clara, CA). Leachates were freeze dried.

Leachates were fractionated following a modification of the method reported in Thurman and Malcolm.¹⁴⁾ The organic carbon was characterized to five fractions: hydrophobic acids and neutrals, and hydrophilic acids, bases and neutrals. Because of insufficient quantities of hydrophobic bases, this fraction was not determined.

2.4 Carbon-13 NMR Spectroscopy

All samples were loaded in the Kel-F holder. Sample weights were approximately 300-500 mg. Solid state carbon-13 NMR spectra were acquired on a GE-300 solid-state Pulse NMR spectrometer with cross polarization and magic angle spinning (CP/MAS). In order to remove side band in spectra, MAS side band elimination software was used. It was equipped with a double tuned, single coil probe with an external lock and an Andrew-Beams type spinning apparatus set for a 1 sec recycle time and a cross polarization time of 1 msec. The magnetic field was 300MHz, 7.0T giving a carbon-13 resonance frequency of 75.46MHz. Typical factors used were: proton 90° pulse, 5 μ ms; contact time, 1msec; recycle delay, 1sec; spinning speed, 5.5KHz; number of scans, 3600-7000.

The carbon-13 NMR analysis was based upon measurement of relative signal areas, with the assumption that all of the carbon in litter and leachates contributes to the car-

bon-13 NMR spectrum. The spectrum can be divided into 8 regions : alkyl(0-50 ppm), methoxyl(50-60 ppm), alcohol(60-65), carbohydrates(65-95 ppm), acetal & aromatic (95-160 ppm), phenolic (160-170 ppm), carboxyl (170-185 ppm), and carbonyl (185-220 ppm) carbons. Typical chemical shifts for the peaks observed in carbon-13 NMR spectra are shown in Table 1.^{11,15,16,17)}

3. RESULTS & DISCUSSION

3.1 Litter

CPMAS carbon-13 NMR spectroscopy allows a semiquantitative interpretation of the

Table 1. Chemical shifts of CP/MAS carbon-13 NMR spectra for various carbon types(Wilson et al., 1981; Steelink and Petsom, 1987; Zech et al., 1987; Frund and Ludeman, 1989; Chen and Robert, 1989; Benner et al., 1990; Newman and Tate, 1991)

Chemical Shift region(ppm)	Assignment
0-110	aliphatic carbon
0-50	saturated alkyl(-CH ₂ -)
50-60	oxygen and nitrogen substituted alkyl in amine and methoxy(-NH, -OCH ₃)
60-65	alcohol(-OH)
65-95	oxygenated alkyl(carbohydrates)
95-160	acetal & aromatic carbon
160-170	phenolic carbon
170-185	carboxylic carbon
185-220	carbonyl carbon

Table 2. Carbon composition of litter, monthly litter leachates(June(JN), July(JL), August(AG), September(SP), and October(OC)), hydrophobic acid(HPO-A) hydrophilic acid(HPI-A), and hydrophilic neutra(HPI-N) determined by carbon-13 NMR

Carbon type(ppm)	Litter	JN	JL	AG	SP	OC	HPO-A	HPI-A	HPI-N
alkyl(0-50)	18.38	23.57	24.57	21.95	15.31	8.66	23.03	14.29	7.57
methoxyl(50-60)	6.68	1.13	1.81	1.57	5.06	4.15	2.23	4.42	3.07
alcohol(60-65)	6.80	0.38	0.37	0.40	2.40	1.89	0.11	1.94	1.72
carbohydrates(65-95)	39.73	21.6	19.9	20.22	21.60	21.23	15.01	21.41	24.15
acetals & aromatic(95-160)	22.42	32.16	33.09	33.78	33.26	37.54	37.65	24.47	37.96
phenolic(160-170)	1.20	4.19	4.44	3.95	5.80	5.50	5.93	5.91	4.95
carboxyl(170-185)	2.68	10.68	11.28	10.73	12.01	13.49	11.26	20.13	12.68
carbonyl(185-220)	1.92	5.51	4.37	4.31	5.22	6.91	4.77	7.24	7.33

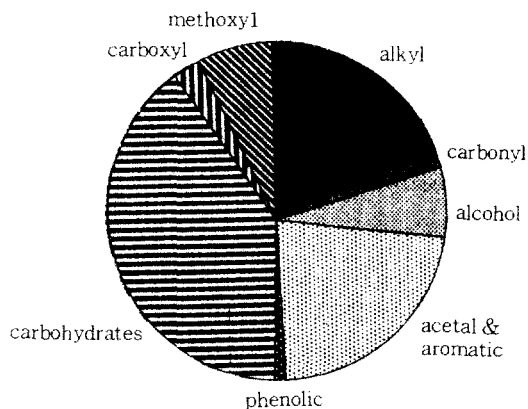


Fig. 1. Various carbon types in litter collected from a northern hardwood forest in central Maine determined by carbon-13 NMR

chemical composition of litter. The spectrum of litter presented in Figure 4 showed strong resonances between 65 and 95 ppm (mainly carbohydrates) dominating with about 40% of total carbon in litter (Table 2). This region originated mostly from ring carbons 1 to 6 of cellulose and hemicellulose monomer units.²¹⁾ The anomeric carbon between 99 and 104 ppm indicated that carbohydrates were linked by O-glycosidic bonding to polysaccharides.

The main carbon fractions in litter were alky, carbohydrates and aromatic carbons, with carbohydrates (Figure 1). Alky carbon (0 to 50 ppm) which had a strong peak at 30 ppm comprised approximately 18 % of carbon. It has long been believed that the majority of organic carbon in litter, especially humic sub-

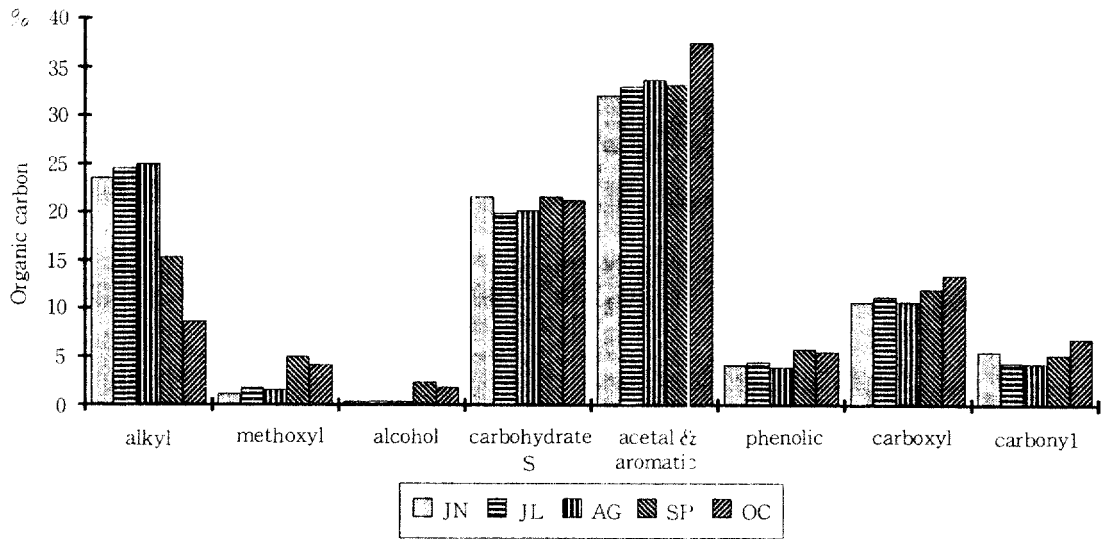


Fig. 2. Various carbon types in monthly collected litter leachates (JN, June ; JL, July ; AG, August ; SP, September ; OC, October) from a northern hardwood forest in Central Maine determined by carbon-13 NMR.

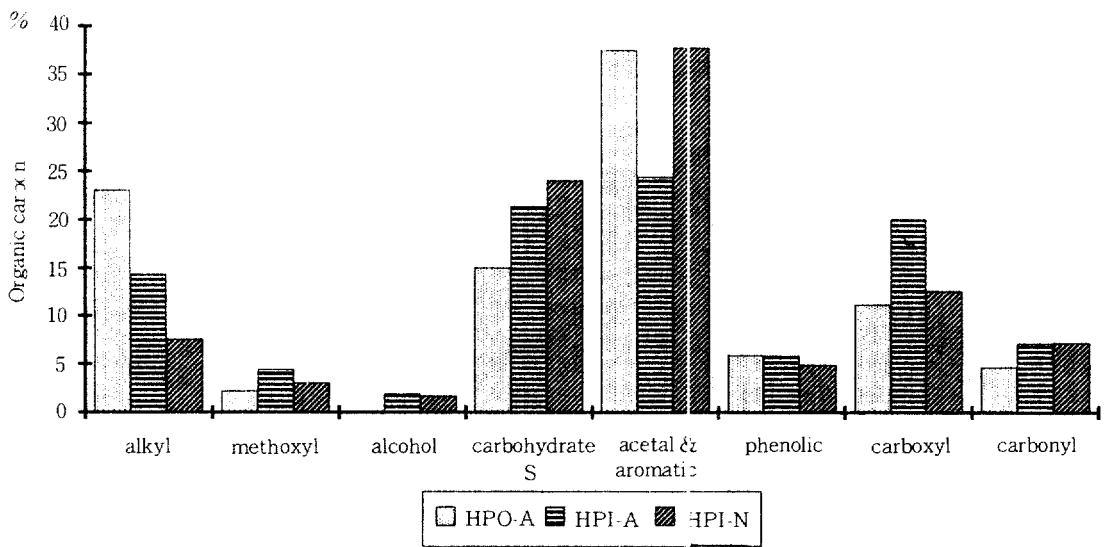


Fig. 3. Various carbon types in hydrophobic acid (HPO-A), hydrophilic acid (HPI-A) and hydrophilic neutral (HPI-N) of litter leachates collected from a northern hardwood forest in central Maine determined by carbon-13 NMR.

stances, consisted of aromatic carbon, but the presence of significant amounts of alkyl carbon has been presented in humic substances.^{22,23} Aromatic carbons were also present, but the absence of prominent peaks at 57 ppm

produced by methoxy carbon indicated that lignin was a minor component in litter, as compared to tannins, which were important non-lignin aromatic components.¹⁷ This fact was confirmed by using dipolar dephased

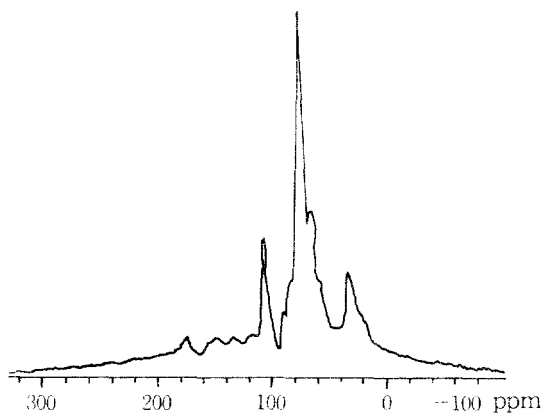


Fig. 4. Carbon-13 NMR spectrum of litter collected from a northern hardwood forest in central Maine.

spectra, suggesting that the peaks at 145 ppm and 154 ppm were derived predominantly from tannin rather than lignin (Figure 4). The strong peak at 30 ppm was assigned to methyl carbon, suggesting the presence of aliphatic compounds. It is thought that this alkyl carbon originates from paraffinic structure derived from microbial lipids²²⁷ and from plant materials such as cutin, suberin, fats and waxes.^{24,251} Carboxyl, carbonyl and phenolic carbons accounted for only 1 to 3 %. As noted by Zech et al.¹⁶¹, decomposition of litter would be accompanied by an increase of aliphatic, carboxyl and carbonyl carbons presumably due to the oxidation of side chains and splitting of lignin molecules.

3. 2 Litter Leachates

Freeze dried litter leachates were analyzed by solid state carbon-13 NMR spectroscopy. The main strong peaks in spectra were present at 30 ppm, 77 ppm, and 167 ppm (Figure 5). The broad peak centered at 30 ppm was assigned to the alkyl carbons including methyl and methylene carbons.¹⁷¹ Primarily, plant or microorganism derived lipids may contribute to this region. Among all organic components of litter, carbohydrates were the most readily available to microorganisms, and compared

with carbohydrates carbon in litter (39.73%), those in litter leachates were very low (approximately 20%) (Table 2). Benner et al.¹⁷¹ examined that the percentages of saturated alkyl carbon and carbohydrates were relatively low compared with leaf materials, whereas aromatic and carboxylic carbons were higher.

There are very significant variation between monthly collections. The collections in June, July and August showed the high content of alkyl carbons (approximately 25%), but these carbons were greatly decreased in the collections of September and October with the lowest alkyl carbons in October collection (8.66 %) (Table 2). In the collections of September and October, methoxyl, aromatic and carboxyl carbons were increased, suggesting the high contribution of microbial degraded lignin to litter composition since lignin was decomposed relatively slowly, which led to the idea that lignin makes an important contribution to the formation of humic substances.^{26,271} Earlier, Melin²⁸¹ proposed that humic substances originate from plant residues, and among the resistant plant materials, lignin could become the core material of humic substance framework by being degraded to simple phenolic monomers, which then undergo oxidative polymerization.²⁹¹ The two most obvious changes in conversion of lignin to humic substances would be a loss of OCH₃ groups by demethylation, producing catechol groups, and oxidation of aldehyde compounds with coupling of intermediates.^{27,29,301}

Among the litter leachate fractions, hydrophobic and hydrophilic acids totaled 90.65% of the organic carbon, with hydrophobic acids being the larger fraction at 68.5% (Table 3). The remaining fractions (neutrals and bases) reached to 9.35%. Hydrophobic acids (HPO-A), and hydrophilic acids (HPI-A) and neutrals (HPI-N) were subjected to carbon-13 NMR analysis for characterization of their chemical composition. Carbon-13 NMR spectra obtained from HPO-A, HPI-A, and HPI-N are shown in Figure 6. The carbon-13 NMR spectrum of hydrophobic neutrals and hydrophilic

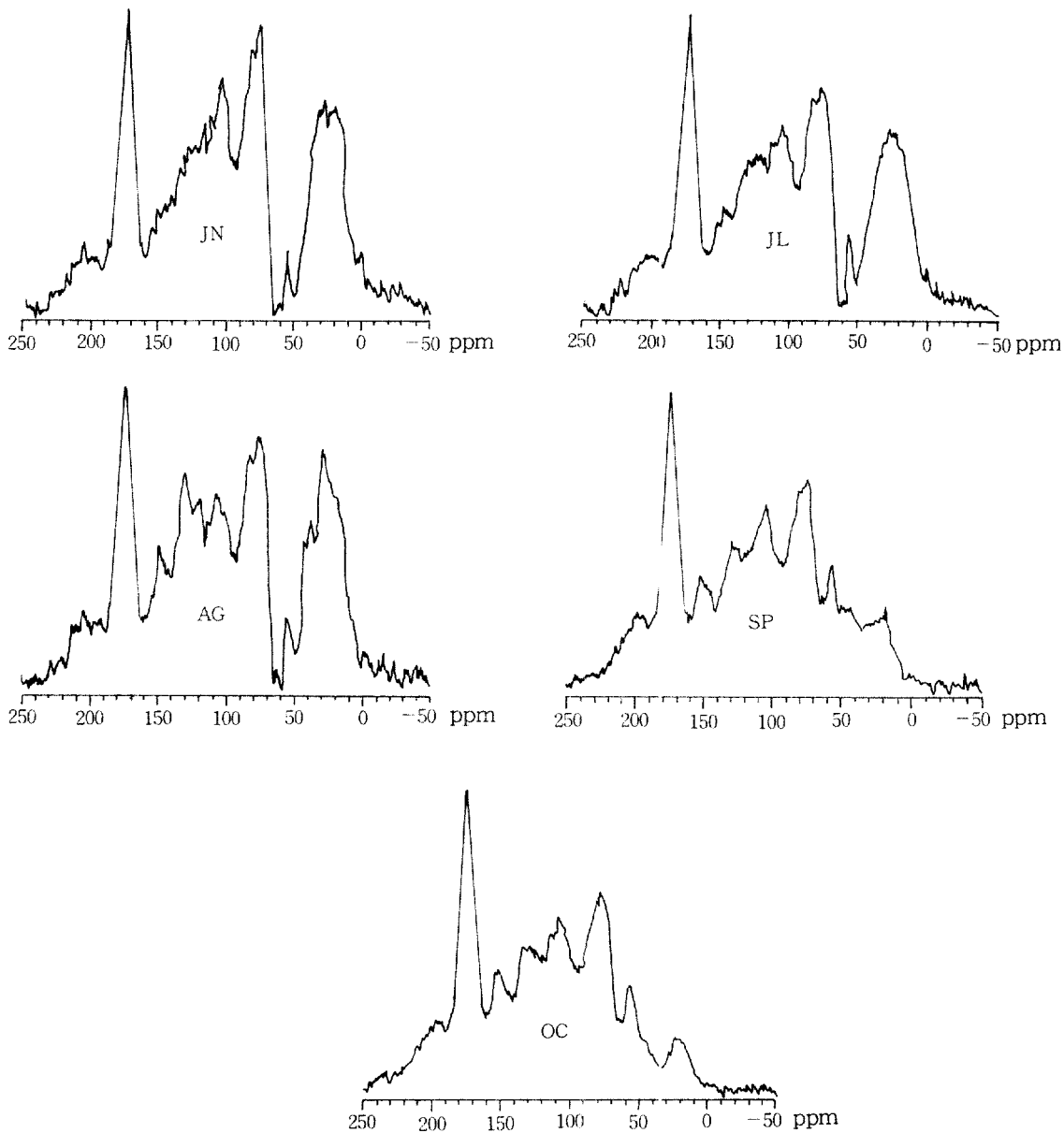


Fig. 5. Carbon-13 NMR spectra of litter leachates (JN, June ; JL, July ; AG, August ; SP, September ; OC, October) collected from a northern hardwood forest in central Maine.

bases could not be recorded due to the low content of organic carbon in samples.

The carbon-13 NMR spectra revealed that there were considerable differences in chemical composition between fractions (Table 2). The hydrophobic acid fraction were mainly a

mixture of alkyl carbon (0-50 ppm), and acetal & aromatic carbon (95-160 ppm) and carboxyl carbon (170-185 ppm). The broad signal between 15 to 50 ppm represents the complex heterogeneity of alkyl carbon including methylene and methyl carbon in long chain and

Table 3. Chemical composition of litter leachates collected from a northern hardwood (HPO, hydrophobic; HPI, hydrophilic; B, base; A, acids; N, neutrals)

	pH	DOC	HPO-A	HPO-N	HPI-B	HPI-A	HPI-N
		ppm	%	%	%	%	%
August	3.62	115.65	68.50	3.72	3.67	22.15	2.96

mass spectra.³¹⁾ The aromatic region between 95 to 160 ppm suggests the presence of lignin structures in the hydrophobic acid fraction. Bands with chemical shifts around 155 ppm originated from phenolic carbons and from nitrogen substituted aromatic carbons.³²⁾ The non-oxygenated aryl carbons, occurring in the range of 110-142 ppm, were mostly responsible for the aromatic carbon content. This fact was supported by the strong peak at 57 ppm which was due to the methoxyl carbon in lignin structures. In this fraction, the contribution of angiosperm (deciduous) lignin to the aromatic carbon may be significant. However the contribution of other aromatics such as lignans, stilbenes, flavonoids and tannins could not be excluded. Differences between hydrophobic and hydrophilic acids in the carbon distribution were not very prominent. The main feature of hydrophilic acid was the exceptional high proportion of carboxyl carbon and low proportion of alkyl and aromatic carbons (Figure 2), suggesting the high degree of microbial oxidation. Chen and Robert¹⁹⁾ investigated the biodegradation of spruce lignin by carbon-13 NMR and concluded that oxidative cleavage of a and b side-chains of the phenyl propane unit was the most important reaction during the fungal degradation of the lignin. The region 170 to 185 ppm represented carboxylic carbons, and the region 185 to 220 ppm represented carbonyl carbons such as aldehyde and ketone carbons.⁹⁾ However, the possibility of the presence of the amide group could not be excluded in this region, and this region may also arise from the spinning side band. The hydrophilic neutrals contained very low alkyl carbon compared with other

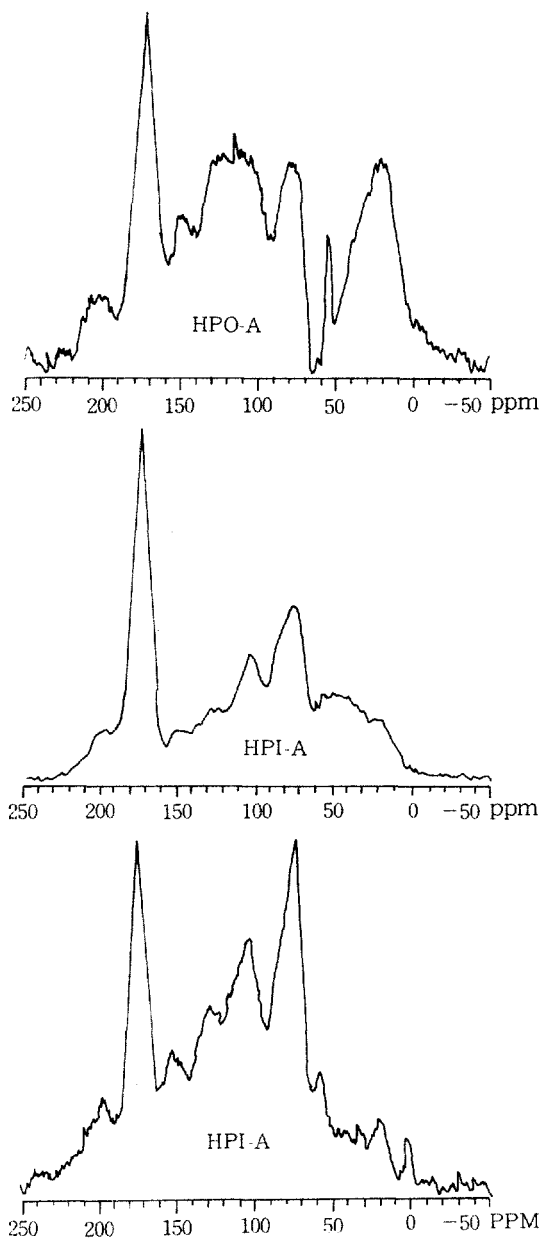


Fig. 6. Carbon-13 NMR spectra of litter leachates fraction (HPO-A, hydrophobic acid; HPI-A, hydrophilic acid; HPI-A, hydrophilic neutral) collected from a northern hardwood forest in central Maine.

branched alkyl chains.¹⁰⁾ n-Alkanes, n-fatty acids, n-alcohols, diols, sterols and n-alkyl esters were found by pyrolysis-field desorption

fractions, and the amounts of carbohydrates, aromatic and carboxyl carbons were very similar.

4. CONCLUSION

The spectrum of litter showed that the main carbons were alkyl carbon derived from plant lipids such as cutin, suberin, fats and waxes, carbohydrates linked by O-glycosidic bonding to polysaccharides and aromatic carbons. The amounts of carboxyl, carbonyl and phenolic carbons were very minimal. However, in litter leachates, alkyl, carbohydrates, aromatic and carbonyl carbons were dominant carbon species due to the microbial degradation of litter. There were very significant variation between monthly collections of litter leachates. The collections in June, July and August showed the high content of alkyl carbons, but these carbons were greatly decreased in the collections of September and October while methoxyl, aromatic and carboxyl carbons were increased due to the high contribution of microbial degraded lignin to litter leachate composition.

Among the litter leachate organic fractions, hydrophobic and hydrophilic acids were dominant organic carbons, with hydrophobic acids being the larger fraction. The spectra of hydrophobic acids (HPO-A), and hydrophilic acids (HPI-A) and neutrals (HPI-N) revealed that there were considerable differences in chemical composition between fractions. The hydrophobic acids were mainly a mixture of alkyl, acetal & aromatic and carboxyl carbons, while hydrophilic acids contained the exceptional high proportion of carboxyl carbon and low proportion of alkyl and aromatic carbons, suggesting the high degree of microbial oxidation. The hydrophilic neutrals contained very low alkyl carbon compared with other fractions.

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