

Ion chromatography에 의한 나뭇잎의 低分子量 有機酸 分析 *1

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Determination of Low Molecular Weight Organic Acids in Pacific Silver Fir Leachates by Using Ion Chromatography *1

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要 約

나뭇잎으로부터 발생하는 저분자량 유기산을 이온크로마토그래피를 이용하여 분석하였다. 이 방법은 자연상태의 나뭇잎 (needle litter)을 유기용매의 추출 없이 그대로 이용할 수 있어서 사용하기에 편리하다. 본 논문에서는 이온 크로마토그래피의 사용방법과 침엽수잎(Pacific Silver Fir)의 부후정도에 따른 유기산의 종류와 농도의 변이를 간략하게 소개한다.

초기의 침엽수잎 여과액으로부터는 C₂에서 C₄까지의 아세트산(acetic acid), 프로피온산(propionic acid), 옥살산(oxalic acid) 그리고 포름산(formic acid) 등이 주종을 이루지만 부후정도가 커짐에 따라 방향족을 가진 방향족 유기산(aromatic organic acid)이 발생한다.

Keywords : Ion chromatography, organic acids, needle leachate

1. INTRODUCTION

Generally, low molecular weight organic acid(LMWOA) is a compound which contains hydroxyl, carboxyl groups in aliphatic chain or phenolic ring. Under natural conditions, the tops and roots of trees, shrubs, grasses, and other native plants annually supply large quantities of organic residue. Organic substances present in forest floor may include those which were exuded from plants, produced during decaying process of plant materials, or produced by microorganisms. Accordingly, it is considered that the kind and amount of organic substances in forest floor are largely influen-

ced by the vegetation.^{1,2)}

Organic acids are derived from intermediate degradation products of lignin, products of oxidative degradation of litter being produced by decomposing microorganisms, and components of the krebs cycle which is the final pathway for conversion of organic material into energy, carbon dioxide, and biosynthetic building blocks.^{3,4)} Organic acids are involved in the solubilization, mobilization, and transport of mineral matter in soil.^{5,6)} Especially, it is interesting that the presence of organic acids in soil can help prevent acidification of streams and creation of elevated dissolved Al concentrations toxic to fish.^{3,7)} Physiologically, the

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role of organic acids in cell division, cell elongation, and the action of growth regulator is not clear, and the relation between organic acid accumulation and production and the role of mycorrhizal fungi have to be examined. In addition, the concentration of each organic acid in each source and what concentration is favored in reaction with mineral are still obscure. Among organic materials, organic acids occur in excreting products from roots⁸⁾ and in leachates from surface litter and needle.^{9,10)}

A considerable input of organic compounds from decomposing needles of pacific silver fir which is a dominant species in the central Cascades, Washington, U. S. A., are deposited on the soil. However, little is known about the amount or types of low molecular weight organic acids released from needle and litter. Therefore, the identification and quantification of LMWOA is essential for understanding their pedogenic role in this area. The purpose of this paper is to present the application of ion chromatography to determine simple organic acids in water leachates of fresh needle and litter.

2. METHODS & PROCEDURES

2.1 Sample Site

The site I have collected samples is the Cascades Mountains of Washington State. The site is at about 1130m (3700 feet) elevation in an old growth stand of pacific silver fir and hemlock. The precipitation here is about 235 cm per year.

2.2 Sample Extraction and Preparation

The cutting sites of needles were sealed with paraffin to prevent the flow of excreting components. LMWOA's were leached from silver fir needles with distilled water for 24 hrs. Simulated decomposed needles were prepared by soaking fresh needles in distilled water at 4°C for 7 days and 150 days.

The extracted samples were filtered with 22 μ m millipore membrane filter. For which are

particularly colored, the samples were neutralized to a pH above 7.0 with dilute NaOH and passed through C-18 Sep-pak to remove hydrophobic organics such as fulvic/humic acids which could poison column. Sometimes chloroform (CHCl₃, 0.8% weight/volume) was added to the filtered samples for protecting organic acids from microbial growth.

2.3 Ion Chromatography

The identification and quantification of LMWOA was performed by ion chromatography utilizing conductivity detector.

Ion chromatography is one type of high performance liquid chromatography (HPLC).¹¹⁾ The ion chromatography system utilized is a Dionex 2010i system (Sunnyvale, CA) which has been updated with the addition of a gradient pump, autoregenerating accessory, eluent degas module, and automatic sample introduction system. A schematic of the setup is shown in Figure 1. The column used were a guard column (MPIC-NG1), precolumn (HPIC-AG5A), separator column (HPIC AS5A-5 μ), and membrane suppressor cartridge (AMMS).

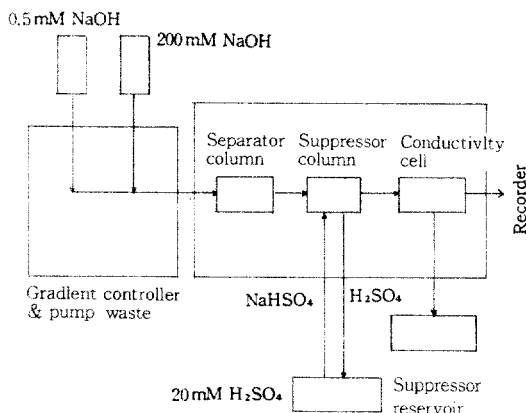


Fig 1. Scheme of ion chromatography

The gradient pump mixes solution from two sources to provide an eluent at 1ml/min (typical psi about 2000) which initially is 0.5 mM NaOH and is progressively increased to

about 100 mM NaOH over the course of a 60 minute run (Table 1). This allows anions which only weakly associated with the exchange resin to be separated early in a given run and as the eluent increases in NaOH concentration, the anions which are bound more strongly with the exchange resin are separated. Therefore, both mono-, di-, and tri-carboxylic acids may be separated, along with inorganic anions (fluoride, chloride, nitrate, carbonate, sulfate, and phosphate).

Table 1. Eluent gradient program

Time (min)	% Eluent 1 * ¹	% Eluent 2 * ²
0	100	0
10	90	10
50	80	20
53	60	40
60	100	0

*¹ : 0.5mM NaOH

*² : 200mM NaOH

Referring to Figure 1 the flow through the chromatography system is as follows. Deionized water is degassed by bubbling He gas through it for 24 hours. Eluent 2 is 200 mM NaOH and prepared by adding 16 ml of 50 % NaOH per liter degassed deionized water. It is imperative that the carbonates in the eluent be kept as low as possible, otherwise the baseline of the chromatograms would drift upward excessively during the chromatography run. It is best to use 50% liquid NaOH to prepare the eluent since any CO₂ which is introduced from the atmosphere will precipitate as Na₂CO₃ will settle out, therefore the 50% NaOH solution should be added by pipet without disturbing any Na₂CO₃ which has settled. Solid NaOH adsorbs CO₂ quickly and is not recommended for eluent preparation. Eluent 1 is 0.5 mM NaOH and is prepared by adding 2.5 ml of eluent 2 per liter degassed deionized water. Both eluents are kept under a He atmosphere to prevent adsorption of atmospheric CO₂.

The eluents are mixed at the gradient pump and the mixture is changed according to Table 1.

After the eluent leaves the gradient pump, anion trap column (ATC) is used to remove and release slowly carbonates or other anions which may be present in the eluents. This helps to maintain a stable baseline of chromatogram.

The sample is introduced by a 150 μ l sample loop. The sample loop may be filled manually by a syringe or is filled from tubes in the autosampler by sucking with a peristaltic pump. In order to inject the sample, the sample loop is brought into line with the eluent flow. The sample and eluent first pass through a guard column (NG-1) which is packed with a non-polar resin to remove hydrophobic organics such as humic and fulvic acids, which could irreversibly adsorb and thereby poison the other column.

A second guard column consists of a short separator column (AG-5A-5) followed by the main separator column (AS5-5). The eluent then passes through the anion micro-membrane suppressor (AMMS). The purpose of suppressor is to exchange protons for cations in the eluent. This will result in the NaOH eluent being converted to H₂O and the anions to their acidic form, thereby resulting in a lower baseline conductivity and a higher signal for some of the anions. The AMMS operates by flowing 20 mM H₂SO₄ through a cation permeable membrane countercurrent to the eluent. The accessory maintains the countercurrent flow at 20 ml /min and also contains a hydrogen-saturated cation exchange resin to regenerate the H₂SO₄ consumed in the AMMS.

Finally the sample is passed through a conductivity cell and the conductivity is measured and automatically corrected for temperature (1.7% per). The stripchart recorder records the time to elute and the conductivity is recorded as peak height. The elution time is utilized to identify the anion and the peak height is used to calculate the concentration, the concentration being determined by ratioing a sample with a standard:

$$\text{Sample concentration} = \left(\frac{\text{Sample peak height}}{\text{Standard peak height}} \right) \times \text{Standard concentration}$$

The concentration of the standard should be near the sample concentration, alternatively a concentration versus conductivity curve may be generated by using multiple standards. The identity of a given peak in a chromatogram is confirmed by spiking the sample with the anion of similar retention time. Further verification may be done by modifying the gradient program and repeating the spiking test.

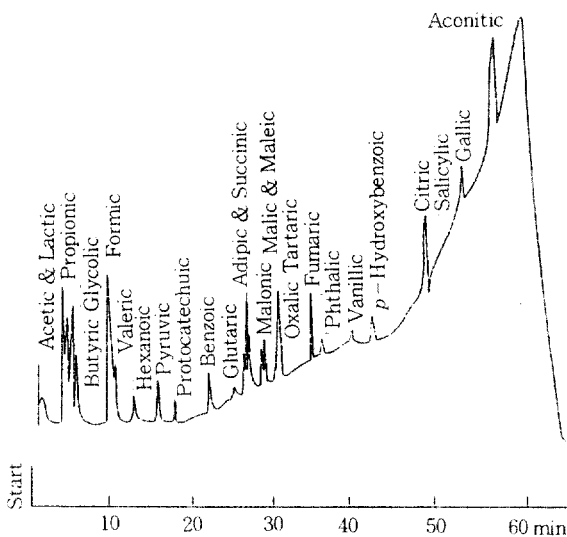


Fig. 2. Low molecular weight organic acids (LMWOA). The concentration of standard organic acids was 1 mg/l.

A standard containing 30 organic acids at a concentration of 1 mg C/l has been run and is shown in Figure 2. The chromatogram shows retention time versus conductivity. The sample is injected at the start point, and this is the starting time for the gradient program given in Table 1.

3. RESULT & DISCUSSION

Ion chromatography is a new liquid chrom-

atographic technique which overcomes the problems of separating and detection of highly acidic and basic ions.¹²⁾ Because of its exceptional selectivity, sensitivity, and speed, ion chromatography is suited to application involving analysis of anions in natural waters. Table 2 and figure 2 show the list of anions which have been successfully separated and detected.

The starting conductivity is about 2.5~3.5 $\mu\text{S}/\text{cm}$ and increases about 4.5~5.5 $\mu\text{S}/\text{cm}$ over throughout the gradient program as is evident in the baseline conductivity. The peaks separate well except for the 4-carbon dicarboxylic acids, and lactic and acetic acids do not separate (Fig. 2). For most of the organics the detection limit is about 0.1 C/l . These acids represent most of the C₁ to C₉ mono- and dicarboxylic acids, both aliphatic and aromatic.

LMWOA's were extracted from fresh and partially decayed silver fir needles (Fig. 3). Initially, leaching with distilled water for 24 hrs yielded only acetic, formic, benzoic and oxalic acids at a concentration of 0.1 to 1.0 mg/l (Table 3). The concentration and diversity of LMWOA leached in distilled water increased with continuing decomposition. After 7 days of leaching, other aliphatic organic acids and aromatic acids were detected at a concentration of 0.5 to 2.0 mg/l: acetic, propionic, butyric, formic, benzoic, succinic, malonic, tartaric, oxalic and vanillic acids (Fig. 4). From litter leachates, McColl and Pohlman³⁾ identified oxalic, citric, aconitic, succinic, fumaric, protocatechuic, p-hydroxybenzoic, vanillic and salicylic acid. Kaurichev et al.¹³⁾ also determined organic acids in water extract from spruce, moss and mixed litter. He represented organic acids by volatile and non-volatile acids, and among the latter, oxalic was invariably present and in the largest quantity. Citric, fumaric, and glycolic acids and, among the volatile acids, formic acid are also found. Smith⁸⁾ found acetic, aconitic, citric, fumaric, malic, malonic, oxalic and succinic acid from hard wood exudates. In the

Table 2. Organic acids characteristics listed in order of elution. The retention time is the time from sample introduction until the anions eluted from the column and detected by the conductivity detector.

Acid	#C	Formula	R. T.	pKa 1	pKa 2	pKa 3
Acetic	2	CH ₃ COOH	7.26	4.75		
Lactic	3	CH ₃ CH(OH)COOH	7.26	3.08		
Propionic	3	CH ₃ CH ₂ COOH	8.25	3.83		
Butyric	4	CH ₃ (CH ₂) ₂ COOH	8.58	4.82		
Formic	1	HCOOH	11.55	3.75		
Valeric	5	CH ₃ (CH ₂) ₃ COOH	11.88	4.82		
Hexanoic	5	CH ₃ (CH ₂) ₄ COOH	14.85			
Protocatechuic	7	3,4-OH AR COOH	18.15			
Benzoic	6	AR COOH	20.79	4.19		
Carbonate		CO ₃	26.00			
Glutaric	5	COOH(CH ₂) ₃ COOH	26.57	3.77	6.08	
Adipic	6	COOH(CH ₂) ₄ COOH	26.90	4.42	5.41	
Succinic	4	COOH (CH ₂) ₂ COOH	26.90	4.16	5.61	
Malonic	3	COOH CH COOH	27.56	2.83	5.69	
Aspartic	4	COOH CH=CH COOH	28.38	1.83	6.07	
Malic	4	COOH CH(OH) CH ₂ COOH	28.38	3.40	5.11	
Tartaric	4	COOH CH(OH) CH(OH) (COOH)	29.04	3.04	4.37	
Oxalic	2	COOH COOH	30.36	1.23	4.19	
Fumaric	4	COOH CH=CH COOH	34.32	3.10	4.60	
Phthalic	8	1,2-COOH AR	36.47	2.89	5.51	
Vanillic	8	3-OH 4-COCH ₃ AR COOH	42.57	4.36		
<i>P</i> -OH benzoic	7	4-OH AR COOH	45.54	4.48	9.32	
Citric	6	COOH CH ₂ C(OH) (COOH) (CH ₂ COOH)	47.36	3.14	4.77	6.39
Salicylic	7	2-OH AR COOH	48.35	2.98	12.38	
Gallic	7	3,4,5-OH AR COOH	49.83	4.41		
Aconitic	6	COOH CO=C(COOH) CH ₂ COOH)	51.28			

gravitational water and capillary water of litter leachates, Vedy and Bruckert ⁴⁾ showed the presence of acetic, formic, lactic, oxalic, malic, citric and these compounds continuously produced from the litter. Likens ¹⁴⁾ and Keene ¹⁵⁾ identified many organic acids in precipitation and mentioned that formic and acetic acids were a major source of acidity in precipitation.

After long leaching (150 days), the concentration increased and the variety and complexity was greater (Fig. 5). Numerous substituted benzoic acid derivatives were produced in 150 days of leaching; benzoic, phthalic, vanillic, and p-hydroxybenzoic acids. Plant cell

walls contain p-coumaric, ferulic and p-hydroxybenzoic acids which are bound to polysaccharides. However, in litter leachates collected by lysimeters, phenolic acids which are degradation products of lignin ^{16, 17)} and may be leached from plant litter ¹⁸⁾ were not easily detected. Whitehead ¹⁹⁾ and Shindo and Kuwatsuka ²⁰⁾ reported the occurrence of p-hydroxy benzoic, vanillic, p-coumaric, ferulic, syringic, salicylic, and protocatechuic acids in most of the soils, but did not detect gallic, caffeic, sinapic acids, resorcinol, and phloroglucinol. Usually, total quantities of the phenolic acids amounted to 0.004-0.009 percent of the total organic matter of the soil, and such small

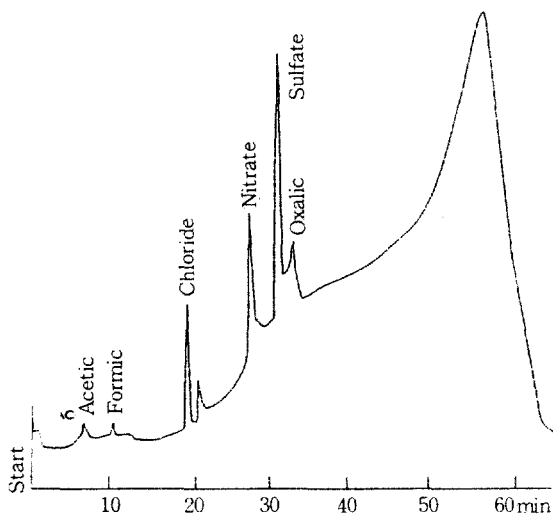


Fig 3. LMWOA extracted from fresh needles after 24 hrs

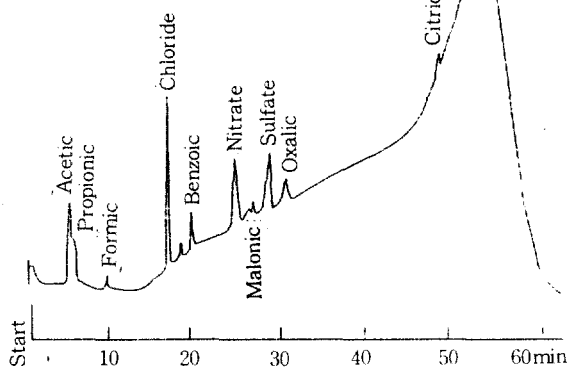


Fig 4. LMWOA extracted from needles decomposed for 7 days

Table 3. Concentration of LMWOA after 24 hrs 7 days, and 150 days decomposition in distilled water (ppm)

	24 hrs	7 days	150 days
Acetic	0.13	0.75	47.90
Propionic		0.76	
Butyric			10.86
Formic	0.05	0.09	2.72
Benzoic		0.85	25.01
Malonic		0.21	47.50
Tartaric			13.33
Oxalic	1.14	0.67	22.50
Fumaric			1.19
Phthalic			35.36
Vanillic			20.83
P-OH			12.50
Benzoic			
Citric		0.12	17.37
Aconitic			3.57

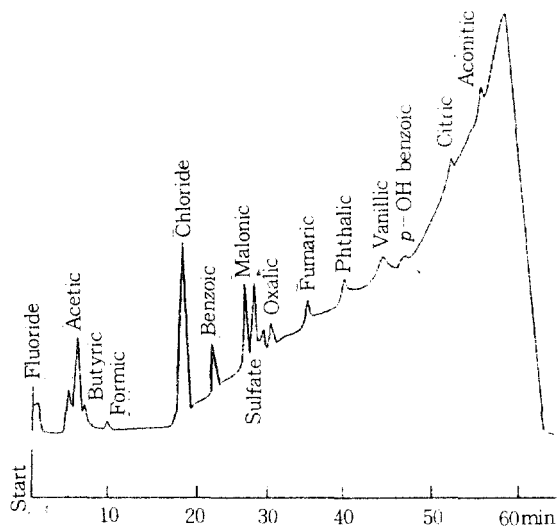


Fig 5. LMWOA extracted from needles ; decomposed for 150 days (25 times dilution)

amount was to be expected in view of the susceptibility of phenolic compounds to microbial attack and possibly to leaching and reaction with other constituents. As noted by Whitehead and Hartley, ²¹⁾ phenolic acids might be

bound to high molecular weight polymer such as humic / fulvic acids after leaching in litter.

These LMWOA may originate from leach

ing of needles and from microbial degradation of dissolved organic matter. Acetic, oxalic, formic, propionic, fumaric and malonic acids are all products of oxidative degradation. Simple phenolic acids (i. e. p-hydroxybenzoic and vanillic) may be intermediate products of lignin degradation.^{16,17)} Phenolic acids are especially important with respect to humus formation. One perplexing characteristic of soluble organic acids leached from needles is that they are short-lived and often can not be easily found in litter leachates. The litter leachates collected by lysimeters does not contain phenolic acids, but litter leachates obtained by centrifugation show the presence of small quantities (0.05 to 1.15 mg /l) of phenolic acids (benzoic, p-hydroxybenzoic and vanillic). Several investigators^{3,5)} have demonstrated that extracts of fresh and decaying leaves have the ability to solubilize, complex, and transport Fe, Al, and other metals in soil. The data showed the presence of several aliphatic acids including acetic, propionic, butyric, formic, malonic, oxalic, fumaric, citric and aconitic acids. Some of these short chain aliphatic acids are reported to form stable 5- and 6-membered ring structures with metals (chelation).²⁰⁾

However, low molecular weight organic acids generally can not be exactly decided whether the isolated acid belongs to the constituents of an organism or whether it is a transformation product.

4. CONCLUSION

1. Ion chromatography is a sensitive and useful analytical technique which has the capability of analyzing for a number of important anions in the leachates of needles and litter.
2. The concentration and diversity of low molecular weight organic acids increases as time of leaching /decomposition increases.
3. Acetic, formic and oxalic acids are leached after 24 hrs from needles in distilled water,

and these aliphatic organic acids increase after 7 days, probably due to long leaching and oxidative degradation.

4. Phenolic acids (i. e. p-hydroxybenzoic and vanillic) appear after 150 days of leaching, suggesting that these low molecular weight organic acids derive from lignin degradation.

REFERENCES

1. Stevenson, F. J. 1967. Organic acids in soil. In *Soil Biochemistry* (A. D. McLaren et al.), Marcel Dekker, New York : 119~145
2. Dewall, D. R., G. C. Ribblett, J. D. Helvey, and J. Kochenderfer. 1985. Laboratory investigation of leachate chemistry from six Appalachian forest floor types subjected to simulated acid rain. *J. Environ. Qual.*, 14(2) : 234~240
3. McColl, J. G. and A. A. Pohlman. 1986. Soluble organic acids and their chelating influence on Al and other metal dissolution from forest soils. 1986. *Water, Air, and Soil Pollution*. vol. 31 : 917~927
4. Vedy, J. C., and S. Bruckert. 1982. Soil solution : Composition and pedogenic significance. In *Constituents and properties of soil*, Academic Press : 185~213
5. Ellis, R. C. 1971. The mobilization of iron by extracts of Eucalyptus leaf litter. *J. Soil Sci.*, 22(1) No. 1 : 8~22
6. Manley, E. P. and L. J. Evans. 1986. Dissolution of feldspars by low molecular weight aliphatic and aromatic acids. *Soil Sci.* vol. 141 : 106~112
7. Hue, N. V., G. R. Craddock, and F. Adams. 1986. Effect of organic acids on aluminum toxicity in subsoils. *Soil Sci. Soc. Am. J.*, vol. 50 : 28~34
8. Smith, W. H. 1976. Character and significance of forest tree root exudates. *Ecology*. vol. 57 : 324~331
9. Millar, C. S. 1974. Decomposition of coniferous leaf litter. In *Biology of plant litter decomposition*, vol. ed. C. H. Dickinson, and

- G. J. F. Pugh, Academic Press, London and New York : 105~112
10. Porter, P. M., W. L. Banwart, and J. J. Hassett. 1986. Phenolic acids and flavonoids in soybean root and leaf extracts. *Environmental and Experimental Botany*, 26(1) No. 1 : 65~73
 11. Gjerde, D. T. and J. S. Fritz. 1987. In Ion Chromatography, 2nd ed. Huthig Verlag, Heidelberg, Basel, New York : 283~290
 12. Rich, W. E., and R. A. Wetzel. 1979. Ion chromatographic analysis of trace ions in environmental samples. In Monitoring toxic substances (D. Schuetzle), ACS symposium Ser., vol. 94 : 233~246
 13. Kaurichev, I. S., T. N. Ivanova and Yem Nozdrunova. 1963. Low molecular organic acid content of water soluble organic matter in soils. *Soviet Soil Sci* : 223~229
 14. Likens, G. E. 1983. The composition and deposition of organic carbon in precipitation. *Tellus*, vol. 35B : 16~24
 15. Keene, W. C., J. N. Galloway, and J. D. Holden. 1983. Measurement of weak organic acidity in precipitation from remote areas of the world. *J. Geophys. Res.* 88, No. C₉ : 5122~5130
 16. Kirk, T. K. 1983. Degradation and conversion of lignocelluloses. In. The filamentous fungi, vol. 4, fungal technology (Smith, J. E., Berry, D. R., and Kristiansen, B.). London : 266~295
 17. Kogel, I., R. Hempfling, W. Zech, P. Hatcher, and H. Schulten. 1988. Chemical composition of the organic matter in forest soils : 1. Forest litter. *Soil Science*, 146(2) : 124~136
 18. Kuiters, A. T., and H. M. Sarink. 1986. Leaching of phenolic compounds from leaf and needle litter of several deciduous and coniferous trees. *Soil Biol. Biochem.*, 18(5) : 475~483
 19. Whitehead, D. C. 1964. Identification of *p*-hydroxybenzoic, vanillic, *p*-coumaric and ferulic acids in soils. *Nature*. vol. 2 : 417~418
 20. Shindo, H., and S. Kuwatsuka. 1978. Behavior of phenolic substances in the decaying process of plants. VIII. Changes in the quality and quantity of phenolic substances in the decaying process of rice straw, ladino clover, and red oak leaves. *Soil Sci. Plant Nutr.*, 24(2) : 221~232
 21. Whitehead, D. C, Hazel Dibb, and R. D. Hartley. 1981. Extractant pH and the release of phenolic compounds from soils, plant roots and leaf litter. *Soil Biol. Biochem.*, vol. 13 : 343~348