

## Determination of Water-Soluble Low-Molecular-Weight Organic Acids in Soils by Ion Chromatography

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Low-molecular-weight organic acids (LOAs) widely occur in soils and affect the soil properties and processes. We developed a simplified and relatively rapid method to identify and quantify the water-soluble LOAs in soils by using ion chromatography with an exclusive column for organic acids, an ion exchange column for anions, and an electric conductivity detector. These combinations enabled to separate more than ten kinds of LOAs. Freeze-drying and passage through Sep-Pak Plus reverse-phase cartridge columns (Waters Corp., Milford, MA) were introduced as pretreatments to concentrate and purify the soil-water extracts. By applying this method, we investigated the vertical distribution of LOAs in two different adjacent soil profiles in Hamatonbetsu, Hokkaido, Japan. One profile consisted of a Sand-dune Podzol (Typic Haplorthods) and the other of a Sand-dune Regosol (Typic Udipsamments). Formic, lactic, oxalic, malic, and citric acids were detected in all the water-extracts from horizon-wise soil samples. In contrast, propionic, butyric, and succinic acids were not detected in any samples. Acetic acid was identified only in the surface horizons. The total amounts of LOAs ranged from 4.7 to 37.2  $\mu\text{mol kg}^{-1}$  with a predominance of formic and oxalic acids. The total amounts of LOAs decreased with the increase in the depth in the Regosol. LOAs were abundant in the spodic Bh and Bs1 horizons as well as in the surface horizon in the Podzol, suggesting that LOAs play a critical role in the podzolization process. The amounts of water-soluble LOAs were closely correlated with the soil acidity. These results indicated that further attention should be paid to the water-soluble LOAs determined by the simplified method using ion chromatography in the present study, in relation to their role in the soil formation processes and soil acidification.

**Key Words:** ion chromatography, low-molecular-weight organic acid (LOA), oxalic acid, podzolization, profile distribution.

Low-molecular-weight aliphatic carboxylic acids, which are commonly designated as low-molecular-weight organic acids (LOAs), widely occur in forest and agricultural soils. They are produced and released in soils during the degradation of organic materials by fungi and bacteria as well as occur in root exudates and leachates from litter layers (Stevenson 1967; Fox 1995). Since the final concentration of LOAs results from a balance between the

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rates of production and degradation in the soil environment, they generally have a short-lived existence in soils and comprise only a small fraction of the total soil carbon and the total dissolved organic carbon in the soil solution (Huang and Violante 1986; Fox 1995). Nevertheless, close attention should be paid to the LOAs, because they markedly affect the soil properties and processes through their strong metal-chelating and ligand-exchange abilities.

Recently, studies using HPLC on LOAs in water-extracts and soil solutions from soils and forest litter have been conducted to analyze their concentration and function, especially in terms of metal dissolution, metal speciation, and phosphate availability, in the soil environment (Pohlman and McColl 1988; Fox and Comerford 1990; Tam and McColl 1991; Tani et al. 1993, 1995; Baziramakenga et al. 1995; Shen et al. 1996, 1997; Tani and Higashi 1997, 1999; van Hees et al. 1999). The HPLC methods for the determination of LOAs include reverse-phase chromatography (RPC) and ion chromatography in ion-exchange and ion-exclusion modes (IEC). IEC in combination with an electric conductivity (EC) detector allows a good separation of several LOAs and a sufficient sensitivity to identify low concentrations of LOAs without interference of a large void peak with tailing compared to RPC and IEC with a UV detector (Baziramakenga et al. 1995; van Hees et al. 1999). Especially, the column for IEC in the ion-exclusion mode provides adequate selectivity and separation for the determination of the LOAs.

In Japan, Tani et al. (1993) developed a method for quantifying LOAs in forest soils and applied this method to several forest soils in Japan such as Andosols, Podzols, Brown forest soils, and Red-Yellow soils (Tani et al. 1995; Tani and Higashi 1997, 1999). They used two types of columns, one for RPC with a UV detector and the other for IEC with an EC detector, which enabled to separate ten kinds of LOAs in soil extracts. However, the running time of HPLC for the analysis of the LOAs and the following clean-up time were very long. Moreover, they treated the soil extracts with polyvinyl pyrrolidone resin and used diethyl ether continuous extraction in a liquid-liquid phase to remove impurities, including colored organic fractions, and to concentrate the soil extracts. These pre-treatments were complicated and required the preparation of a custom-made apparatus. These shortcomings should be alleviated to obtain more rapid information about the distribution and functions of LOAs in the soil environment.

The objective of the present study was to develop a simplified and relatively rapid method to identify and quantify the water-soluble LOAs in soils by using ion chromatography in combination with freeze-drying and passage through reverse-phase cartridge columns to concentrate and purify the soil-water extracts. We also report the vertical distribution of LOAs in two different adjacent soil profiles, one consisting of a Sand-dune Podzol and the other of a Sand-dune Regosol, by applying these methods to the determination of the LOAs in horizon-wise soil samples.

## MATERIALS AND METHODS

**Soil samples.** The soil samples used in this study were collected horizon-wise from two profiles in the reserve area of Hokkaido Prefectural Tenpoku Agricultural Experiment Station (N 45°7', E 142°22') in Hamatonbetsu, Hokkaido, Japan. The vegetation consisted of a mixed conifer and deciduous broad-leaved forest (*Abies sachalinensis* and *Betula ermani*) with an understory of bamboo and various mosses. The height of the trees was relatively low probably due to the strong wind. The profiles were located in sand dunes on the seashore

and were about 600 m apart. One soil profile, developed on a micro-depression consisted of Sand-dune Podzols (Typic Haplorthods; Soil Taxonomy 1998), with an A horizon and a thick spodic horizon above and under an E horizon, respectively. The other, developed on a micro-ridge, consisted of Sand-dune Regosols (Typic Udipsamments; Soil Taxonomy 1998), which lacked a distinctive B horizon. The Sand-dune Podzol had a thicker O horizon (5 cm) than the Sand-dune Regosol (2–3 cm), mainly due to the difference in the micro-relief and the subsequent moisture conditions. Major macro-morphological characteristics are described in Table 1. The two profiles were characterized by a weakly developed structure and a very coarse texture. The hardness of the spodic horizons (Bh, Bhs, Bs1, and Bs2 horizons) was medium to compact, while that of the other horizons was very loose to loose. In the preliminary experiment, the surface soil sample (0–13 cm) of a Wet Andosol (Hydric Pachic Melanudands), located at the Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan, was used to examine the applicability of the determination method of LOAs developed in this study. This sample showed a coarse texture and was rich in humic organic matter (Table 1). Field-moist soil samples were passed through a 2 mm pore-size-sieve after removal of the roots or litter as much as possible, and stored at 4°C until extraction.

**Determination of water-soluble LOAs.** Field-moist soil samples, equivalent to 50 g oven-dry soils, were placed in polypropylene vessels (500 mL). Ultra-pure water was used to obtain the water-soluble and free LOA fractions. Ultra-pure water was added to each vessel so as to obtain a combined total volume of added water and moisture in the soil samples reaching 250 mL (solid : water ratio is 1 : 5). After shaking for 1 h with a reciprocating shaker, the suspensions were centrifuged at  $10,000 \times g$  for 20 min and filtered through a  $0.025 \mu\text{m}$  membrane filter (Millipore type VS). Single extraction was applied in the present study.

The water-extract was then concentrated by freeze-drying; 150 or 100 mL of the supernatant was placed in pear-shaped flasks (300 mL) and alkalized with 0.2 mL of  $0.5 \text{ mol L}^{-1}$  NaOH to avoid the evaporation of volatile LOAs. After freeze-drying, the residue was

**Table 1.** Major macro-morphological characteristics of the profiles and the soil samples.

Horizon	Depth (cm)	Moist colour <sup>a</sup>	Field texture <sup>b</sup>	Structure <sup>c</sup>	Hardness <sup>d</sup>	Field moisture <sup>e</sup>
Sand-dune Podzol						
A	0–8	7.5YR 3/2.5	SL	VW FM GR	VL	MD
E	8–15	10YR 5/3	S	VW FI SB	VL	MD
Bh	15–22	7.5YR 4/5	S	VW FI SB	M	MD
Bhs	22–34	5YR 3/4	S	WE FM SB	C	MD
Bs1	34–46	10YR 4/6	S	WE FM SB	M	MD
Bs2	46–60	10YR 5/6	S	WE FM SB	M	MM
C	60–110	10YR 5/4	S	WE FM SB	L	MM
Sand-dune Regosol						
A	0–6	10YR 3/3	S	VW FM GR	VL	MD
BC	6–20	7.5YR 4/4	S	VW FI SB	L	MD
C1	20–30	10YR 4/6	S	MA	L	MD
C2	30–70	10YR 3.5/4	S	MA	L	MD
Wet Andosol						
A1	0–13	7.5YR 2/1	L	WE FM GR	VL	MM

<sup>a</sup>Based on Munsell colour chart. <sup>b</sup>S, sand; SL, sandy loam; L, loam. <sup>c</sup>VW, very weak; WE, weak; FI, fine; FM, fine medium; GR, granular; SB, subangular blocky; MA, massive. <sup>d</sup>VL, very loose; L, loose; M, medium; C, compact. <sup>e</sup>MD, moderately dry; MM, moderately moist.

**Table 2.** Analytical conditions of HPLC.

	TSKgel OApak-A and -P <sup>a</sup>	TSKgel IC-ANION-PW <sub>XL</sub>
Column type	Exclusive column for organic acids	Ion exchange column for anions
Column size	7.8 mm ID × 300 mm × 2 <sup>b</sup>	4.6 mm ID × 35 mm
Eluate	0.75 mmol L <sup>-1</sup> H <sub>2</sub> SO <sub>4</sub>	Borate buffer (pH 7.8) <sup>c</sup>
Flow-rate	1.0 mL min <sup>-1</sup>	1.2 mL min <sup>-1</sup>
Oven temp.	40°C	40°C
Detector	Electric conductivity	Electric conductivity
Injection vol.	0.02 mL	0.1 mL

<sup>a</sup>With guardcolumn TSKgel OApak-P. <sup>b</sup>Direct connection of two columns. <sup>c</sup>Borate buffer with potassium gluconate, acetonitrile, and glycerine.

re-dissolved in 2.5 mL of ultra-pure water by using ultrasonication. The concentrated solution was passed through a cation-exchange cartridge column Sep-Pak Plus Accell CM (Waters Corp., Milford, MA) to remove excess cations and a reverse-phase cartridge column Sep-Pak Plus C<sub>8</sub> or Sep-Pak Plus tC<sub>18</sub> (Waters Corp.) to remove colored hydrophobic organic matter fractions. The cation-exchange cartridge column and the reverse-phase cartridge column were conditioned beforehand with 5 mL of 0.05 mol L<sup>-1</sup> HCl and methanol, respectively. The columns were then washed with 5 mL of ultra-pure water 2 times and replaced by 10 mL of air 5 times. Two types of the reverse-phase cartridge columns were selected based on the degree of contamination with colored organic matter in the concentrated solution.

LOAs were identified and quantified by ion chromatography. The ion chromatograph used in the present study belonged to the TOSOH series 8020 (TOSOH Corp., Tokyo) and was equipped with a degassing module, a pump, a column oven, and an electric conductivity detector. The columns were also purchased from TOSOH Corp. for which the analytical conditions are shown in Table 2. Nine kinds of LOAs, namely formic acid, acetic acid, propionic acid, butyric acid, lactic acid, fumaric acid, succinic acid, malic acid, and citric acid were separated with an exclusive column for organic acids, TSKgel OApak-A (7.8 mm ID × 300 mm, two columns connected directly) and a guard column, TSKgel OApak-P. Oxalic acid was separated with an ion exchange column for anions, TSKgel IC-ANION-PW<sub>XL</sub> (4.6 mm ID × 35 mm) and a guard column, TSKguardcolumn IC-AS. Each peak of LOAs was identified by comparing the retention times of high-grade chemical standard solutions of known LOAs and the concentration was determined by comparing the peak areas of the unknown sample solution with those of known LOA standard solutions. Standards were subjected to the same procedures as those for the soil samples. The pre-examined recovery ratios of each LOA were taken into account in expressing the results as  $\mu\text{mol LOA kg}^{-1}$  dry soil.

## RESULTS AND DISCUSSION

### Chromatographic procedures

The ion chromatogram of the mixture solution of LOA standards by using the exclusive column for organic acids (TSKgel OApak-A) is shown in Fig. 1. It was difficult to separate oxalic acid, but the remaining nine LOAs could be well separated. The total duration of the analysis was about 50 min. The LOAs, which showed lower  $pK_a$  values and more hydrophilic properties, were eluted faster in this column. Although data are not shown, other LOAs in addition to the ten kinds of LOAs analyzed in the present study, namely pyruvic

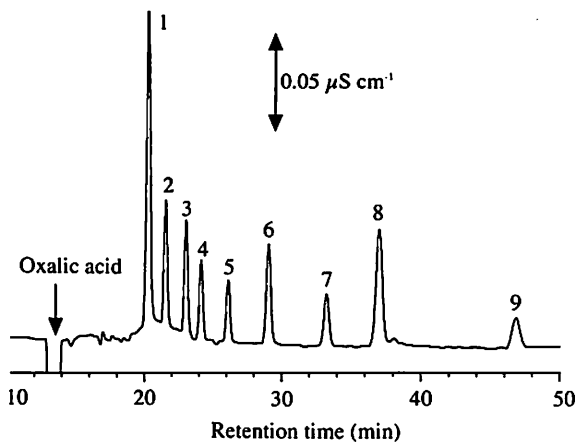


Fig. 1. HPLC chromatogram of standard solution of low-molecular-weight organic acids by using an exclusive column for organic acids (TSKgel OApak-A). Peaks: 1, citric acid; 2, malic acid; 3, lactic acid; 4, formic acid; 5, acetic acid; 6, succinic acid; 7, propionic acid; 8, fumaric acid; 9, butyric acid.

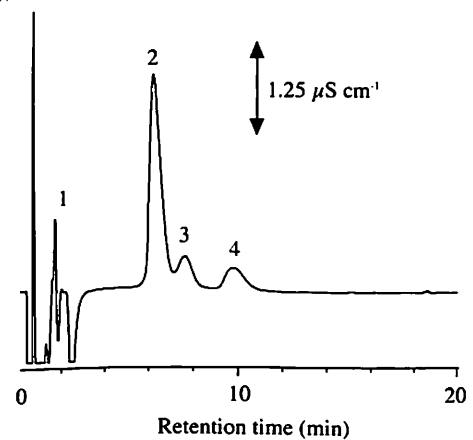


Fig. 2. HPLC chromatogram of standard solution of low-molecular weight organic acids by using an ion-exchange column for anions (TSKgel IC-ANION-PW<sub>x1</sub>). Peaks: 1, formic, acetic, propionic, lactic, and citric acids; 2, succinic and malic acids; 3, oxalic acid; 4, fumaric acid.

acid, 2-oxoglutaric acid, maleic acid, tartaric acid, malonic acid, glutaric acid, itaconic acid, and adipic acid, could be well separated, except for fumaric acid and *t*-aconitic acid. Oxalic acid, which must be one of the predominant LOAs in soils (Fox and Comerford 1990; Tani et al. 1993; Tani and Higashi 1999), appeared in the water-dip of the injected solution and could not be easily identified in the chromatogram.

Meanwhile, oxalic acid was successfully separated by using the anion exchange column as shown in Fig. 2. The total duration of the analysis was about 20 min. It was confirmed that there was no interference with peaks of inorganic anions, such as nitrate, phosphate, and sulfate. Since fumaric acid was also well separated with this anion-exchange column, the problem of the overlapping peak of fumaric acid and *t*-aconitic acid in using the exclusive column for organic acids could be solved.

Ion chromatography in ion-exchange or ion-exclusion modes coupled with the conductivity detector is known to provide a sensitive and rapid measurement of a wide variety of organic compounds including LOAs in soils and soil solutions (Baziramakenga et al. 1995; Shen et al. 1996; van Hees et al. 1999). As mentioned above, the use of two types of ion chromatography columns enabled to separate more than ten kinds of LOAs in the present study.

### Concentration and purification of soil-water extract

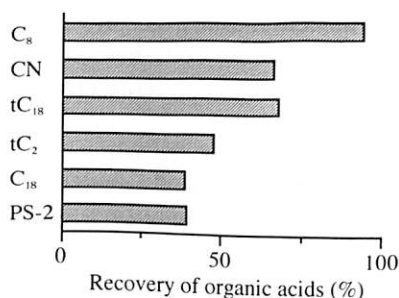
Since the concentration of LOAs in the soil-water extract is extremely low, some procedures to concentrate the solution are critical. Although the solvent extraction methods in a liquid-liquid phase, where diethyl ether or ethyl acetate was commonly used, has been often applied (Tani et al. 1993; Baziramakenga et al. 1995; Shen et al. 1997), it took a long time and the recovery of some LOAs was too low. In the present study, the freeze-drying technique was used. This method is simple and loss due to volatilization can be avoided if the solution is alkalized. However, in contrast to the solvent extraction methods, impurities,

especially colored hydrophobic organic fractions, are also encountered in the concentration process during the freeze-drying and re-dissolving procedures.

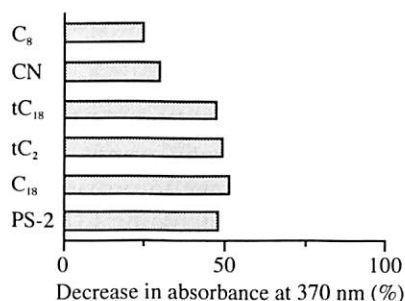
Jinap and Dimick (1991) had examined low-molecular-weight organic acids in roasted cocoa beans by using ion exclusion chromatography and they successfully removed phenolic compounds by passing the water-extracted samples through a Sep-Pak Plus C<sub>18</sub> reverse-phase cartridge column. We tried to remove the hydrophobic organic fraction including phenolic compounds and purify the concentrated solution by using reverse-phase cartridge columns. Six types of cartridge columns were purchased from Waters Corp. and their functional groups were as follows: Sep-Pak Plus C<sub>8</sub>, -Si(CH<sub>3</sub>)<sub>2</sub>C<sub>8</sub>H<sub>17</sub>; Sep-Pak Plus CN, -Si(CH<sub>3</sub>)-(CH<sub>2</sub>)<sub>3</sub>CN; Sep-Pak Plus tC<sub>18</sub>, -SiC<sub>18</sub>H<sub>37</sub>; Sep-Pak Plus tC<sub>2</sub>, -SiC<sub>2</sub>H<sub>5</sub>; Sep-Pak Plus C<sub>18</sub>, -Si(CH<sub>3</sub>)<sub>2</sub>C<sub>18</sub>H<sub>37</sub>; Sep-Pak Plus PS-2, styrene-divinylbenzene copolymer.

Averaged recovery of LOAs after passage of the standard mixture solution through these columns is shown in Fig. 3. The recovery was highest, 94.2%, when the Sep-Pak Plus C<sub>8</sub> column was used. Passage through the Sep-Pak Plus C<sub>18</sub> and Sep-Pak Plus PS-2 columns led to recoveries of approximately 40% and propionic acid, butyric acid, oxalic acid, and succinic acid were mostly adsorbed onto the resin. The recoveries on the Sep-Pak Plus CN and tC<sub>18</sub> columns were 67–68% and were satisfactory. The effectiveness in removing colored organic fractions was estimated by examining the degree of decrease in absorbance at 370 nm for a concentrated water-extract from a Podzolic Bh horizon soil sample after passage through the reverse phase cartridge columns (Fig. 4). The rate of decrease, which ranged from 25 to 51%, was highest in the Sep-Pak Plus C<sub>18</sub> column and lowest in the Sep-Pak Plus C<sub>8</sub> column.

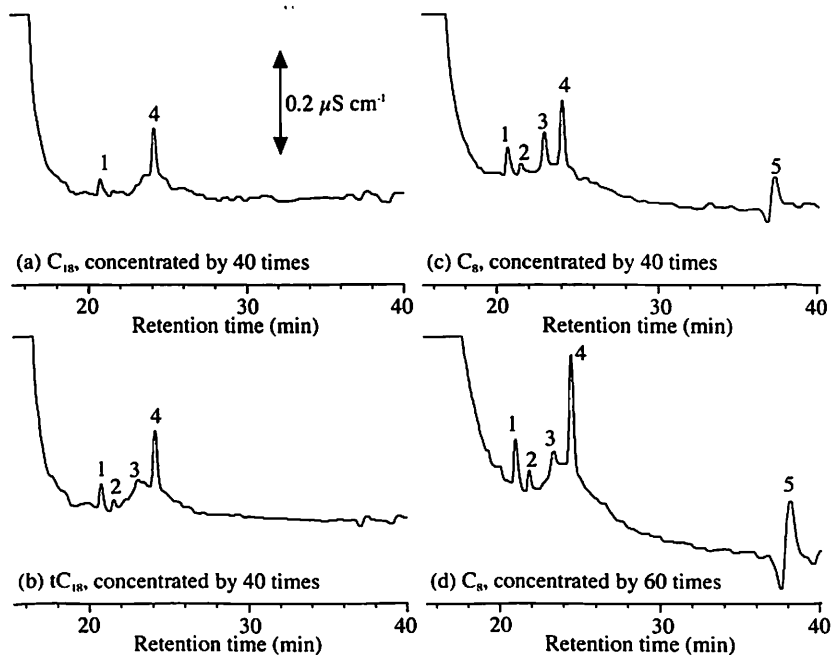
We made another attempt to compare the chromatograms of the concentrated water-extract, which was obtained from a soil sample of the surface horizon of the Wet Andosol (Hydric Pachic Melanudands), by passing the concentrated samples through three types of reverse-phase cartridge columns: Sep-Pak Plus C<sub>8</sub>, which gave the highest recovery of LOAs and showed the lowest effectiveness in removing the colored organic fractions; Sep-Pak Plus



**Fig. 3.** Averaged recovery of low-molecular-weight organic acids (formic, acetic, propionic, butyric, lactic, oxalic, fumaric, succinic, malic, and citric acids) after passage through reverse-phase cartridge columns. Cartridge columns and their functional groups: Sep-Pak Plus C<sub>8</sub>, -Si(CH<sub>3</sub>)<sub>2</sub>-C<sub>8</sub>H<sub>17</sub>; Sep-Pak Plus CN, -Si(CH<sub>3</sub>)(CH<sub>2</sub>)<sub>3</sub>CN; Sep-Pak Plus tC<sub>18</sub>, -SiC<sub>18</sub>H<sub>37</sub>; Sep-Pak Plus tC<sub>2</sub>, -SiC<sub>2</sub>H<sub>5</sub>; Sep-Pak Plus C<sub>18</sub>, -Si(CH<sub>3</sub>)<sub>2</sub>C<sub>18</sub>H<sub>37</sub>; Sep-Pak Plus PS-2, styrene-divinylbenzene copolymer. All the columns were purchased from Waters Corp.



**Fig. 4.** Decrease in absorbance at 370 nm of concentrated water-extract of Podzolic Bh horizon soil sample after passage through reverse-phase cartridge columns. The types of columns and their functional groups are the same as those in Fig. 3.



**Fig. 5.** HPLC chromatograms of concentrated water-extract from A1 horizon of a Wet Andosol. The concentrated water-extract was passed through (a) Sep-Pak Plus  $C_{18}$  column, (b) Sep-Pak Plus  $tC_{18}$  column, and (c) Sep-Pak Plus  $C_8$  column. Water-extract was concentrated by (a)–(c) 40 times and (d) 60 times by freeze-drying. Peaks: 1, citric acid; 2, malic acid; 3, lactic acid; 4, formic acid; 5, fumaric acid.

$C_{18}$ , which gave the lowest recovery and showed the highest effectiveness; and Sep-Pak Plus  $tC_{18}$ , which showed intermediate level in these properties between the columns (Fig. 5). When the water-extract was concentrated by 40 times by freeze-drying and passed through the Sep-Pak Plus  $C_8$  column, five peaks of LOAs, which consisted of citric acid, malic acid, lactic acid, formic acid, and fumaric acid, were identified (Fig. 5c). In contrast, only two peaks and four peaks were detected by using Sep-Pak Plus  $C_{18}$  and Sep-Pak Plus  $tC_{18}$ , respectively (Fig. 5a and b). The concentration of the water-extract by 60 times enhanced the sensitivity of detection as indicated by the increase in the peak area of each LOA, although the effect of tailing in the void peak became more conspicuous (Fig. 5d).

Consequently, the reverse-phase cartridge columns were effective to remove the colored organic fractions and the combination of the ion-exclusion and ion-exchange columns used in the present study with freeze-drying enabled to identify the LOAs in the soil-water extracts. The use of the Sep-Pak Plus  $C_8$  cartridge column or Sep-Pak Plus  $tC_{18}$  cartridge column and concentration of the soil-water extract by 40 times or 60 times depending on the degree of contamination with colored organic matter in the concentrated solution and the concentration of LOAs in the soil-water extracts should be adequate.

### Low-molecular-weight organic acids in soil water-extracts

The soil-water extracts obtained from the horizon-wise soil samples in the profiles of the Sand-dune Podzol and the Sand-dune Regosol were concentrated by 60 times and passed through a Sep-Pak Plus  $C_8$  cartridge column as mentioned above, prior to the ion chromatography analysis of LOAs. Formic acid, lactic acid, oxalic acid, malic acid, and citric acid

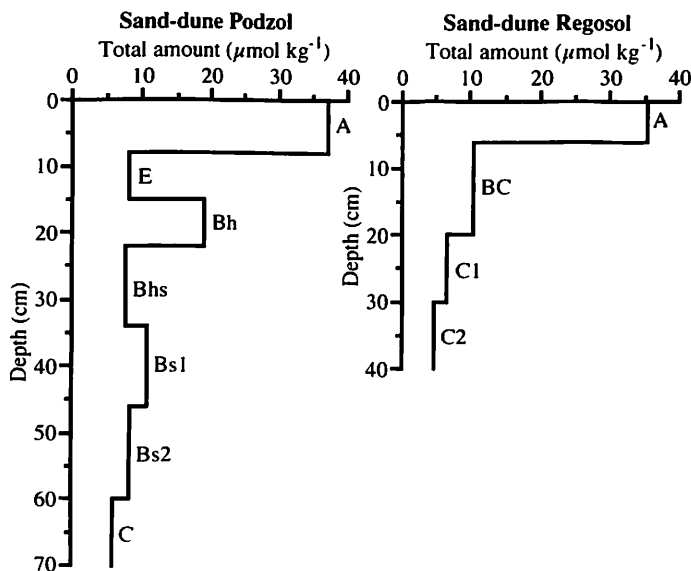
were detected in all the water-extracts (Table 3). Acetic acid was also detected and quantified only in the surface A horizon of the two profiles. In the surface horizons, acetic acid was predominant and reached a concentration of  $14.7 \mu\text{mol kg}^{-1}$  in the Sand-dune Podzol and  $11.9 \mu\text{mol kg}^{-1}$  in the Sand-dune Regosol. Propionic acid, butyric acid, and succinic acid were not detected in any of the samples. In the LOAs, formic acid and oxalic acid predominated, as reported by Fox and Comerford (1990) in the soil solution of Spodosols on the Lower Coastal Plain of Florida, USA, and accounted for 45 to 76% of the total LOAs identified.

The total amounts of LOAs ranged from 5.8 to  $37.2 \mu\text{mol kg}^{-1}$  and from 4.7 to  $35.3 \mu\text{mol kg}^{-1}$  in all the horizons from the Sand-dune Podzol and the Sand-dune Regosol profiles, respectively (Fig. 6). The total amounts of LOAs were highest in the surface A horizons of both profiles, presumably due to the leaching from forest litter layers and high biological activities (Pohlman and McColl 1988; Tam and McColl 1991). Although there

**Table 3.** Amounts of water-soluble low-molecular-weight organic acids in the soil profiles studied ( $\mu\text{mol kg}^{-1}$ ).

	Sand-dune Podzol							Sand-dune Regosol			
	A	E	Bh	Bhs	Bs1	Bs2	C	A	BC	C1	C2
Formic acid	8.37	1.79	4.99	1.79	2.86	2.48	2.16	9.28	3.63	1.47	1.43
Acetic acid	14.7	Tr. <sup>a</sup>	Tr.	ND <sup>b</sup>	Tr.	Tr.	Tr.	11.9	Tr.	ND	ND
Propionic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Butyric acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lactic acid	3.05	1.31	4.03	1.31	2.68	1.88	1.29	1.16	1.49	2.29	1.41
Oxalic acid	8.40	4.33	8.08	3.87	4.04	3.07	2.00	9.98	4.16	2.09	1.05
Fumaric acid	0.22	Tr.	ND	ND	ND	ND	Tr.	ND	ND	ND	ND
Succinic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Malic acid	1.29	0.32	0.90	0.32	0.63	0.46	0.30	1.48	0.53	0.47	0.63
Citric acid	1.12	0.29	0.88	0.29	0.44	0.32	0.06	1.43	0.48	0.28	0.14

<sup>a</sup> Tr., trace amount; <sup>b</sup> ND, no peak detected.



**Fig. 6.** Vertical distribution of the total amounts of water-soluble low-molecular-weight organic acids in the soil profiles of the Sand-dune Podzol (left) and the Sand-dune Regosol (right).



was no distinctive difference in the range of the total amounts of LOAs between the two profiles, the vertical distribution patterns of the total LOAs in the two profiles were markedly different. In the Sand-dune Regosol profile, the total amounts of LOAs decreased with the increase in depth. On the other hand, the amounts of total LOAs were lower in the eluvial E horizon and higher in the spodic Bh and Bs1 horizons of the Sand-dune Podzol profile. Tani and Higashi (1999) also observed that the total amounts of LOAs were larger in the spodic B horizons than in the E horizon of the Typic Podzolic soil derived from greywacke in the subalpine climatic zone. Oxalic acid was the most abundant LOA in these horizons (Table 3), suggesting that such LOAs which act as strong metal-binding ligands are crucial for the podzolization process in the transport of aluminum, iron, and silicates to the lower horizons (Lundström 1993; Lundström et al. 1995; van Hees et al. 1996).

The mobilization of metals such as aluminum and iron by the LOAs is generally recognized to be a dominant feature of many forest soils (Fox 1995). They affect the soil properties and metal dissolution by chelating metals in solution and ligand exchange at mineral surfaces (Stumm 1986). Although LOAs such as oxalic acid and citric acid, which

Table 4. Some chemical properties of the soil-water extracts and soil samples.

Horizon	Depth (cm)	pH <sup>a</sup>	Loss on ignition <sup>b</sup> (g kg <sup>-1</sup> )
Sand-dune Podzol			
A	0-8	5.38	62.7
E	8-15	5.68	17.7
Bh	15-22	5.69	43.0
Bhs	22-34	5.69	32.6
Bs1	34-46	5.72	17.6
Bs2	46-60	5.82	13.7
C	60-110	5.86	9.23
Sand-dune Regosol			
A	0-6	5.12	56.3
BC	6-20	5.78	22.4
C1	20-30	6.06	15.0
C2	30-70	5.80	13.5

<sup>a</sup>pH of the soil-water extract. <sup>b</sup>The weight loss was determined upon heating for 5 h at 550°C.

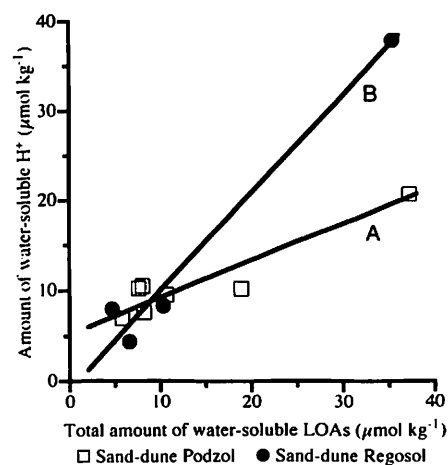


Fig. 7. Correlation of total amounts of water-soluble LOAs with the amounts of water-soluble hydrogen ion ( $H^+$ ) calculated from the pH of the soil-water extract. The lines drawn in this figure represent the reduced major axis (Webster 1997). The lines A and B represent the functional relations between the total amounts of LOAs and hydrogen ion concentrations of the Sand-dune Podzol and the Sand-dune Regosol, respectively.

have low dissociation constants, should also exhibit the acidic effect through proton release (Tan 1986), their effects on the soil acidic properties have not been well elucidated. The pH of the soil-water extracts was lower in the A horizons, where the total amounts of water-soluble LOAs were higher, than in other horizons (Table 4). When the relation between the total amounts of LOAs and hydrogen ion concentrations calculated from the pH was examined, a significant positive correlation was recognized in each profile ( $r=0.935$ ,  $p<0.01$  in the Sand-dune Podzol;  $r=0.985$ ,  $p<0.05$  in the Sand-dune Regosol) as shown in Fig. 7. The slope of the reduced major axis was such that the water-soluble LOAs could be considered to be the principal source of protons. We suggest that the increase in the total amounts of water-soluble LOAs could be attributed to the increase in the soil acidity and associated chemical reactions in the sandy soils studied here.

A highly positive correlation was also found between the total amounts of LOAs and the loss on ignition of an air-dry soil sample ( $r=0.916$ ,  $p<0.01$  in the Sand-dune Podzol;  $r=0.999$ ,  $p<0.01$  in the Sand-dune Regosol), suggesting that the LOAs display a critical interaction with soil organic matter including high-molecular-weight compounds.

These results indicate that close attention should be paid to the LOAs obtained by the simplified method using ion chromatography in the present study, in relation to the soil formation processes and soil acidification.

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