

Chemical Structural Features of Humic-like Substances (HULIS) in Urban Atmospheric Aerosols Collected from Central Tokyo with Special Reference to Nuclear Magnetic Resonance Spectra

Naoya Katsumi^{1),2),*}, Shuhei Miyake²⁾ and Hiroshi Okochi²⁾

¹⁾Department of Environmental Science, Faculty of Bioresources and Environmental Sciences, Ishikawa Prefectural University, 1-308, Suematsu, Nonoichi, Ishikawa 921-8836, Japan

²⁾Department of Resources and Environmental Engineering, School of Creative Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku, Tokyo 169-8555, Japan

*Corresponding author. Tel: +81-076-227-7470, E-mail: n-katsu@ishikawa-pu.ac.jp

ABSTRACT

We measured ¹H and ¹³C nuclear magnetic resonance (NMR) spectra of Humic-like substances (HULIS) in urban atmospheric aerosols isolated by diethylaminoethyl (DEAE) and hydrophilic-lipophilic balance (HLB) resin to characterize their chemical structure. HULIS isolated by DEAE resin were characterized by relatively high contents of aromatic protons and relatively low contents of aliphatic protons in comparison with HULIS isolated by HLB resin, while the contents of protons bound to oxygenated aliphatic carbon atoms were similar. These results were consistent with the results of the ¹³C NMR analysis and indicate that hydrophobic components were more selectively adsorbed onto HLB, while DEAE resins selectively retained aromatic carboxylic acids. Furthermore, we demonstrated that the chemical structural features of HULIS were significantly different between spring and summer samples and that these disparities were reflective of their different sources. The estimated concentrations of HULIS in spring were found to be regulated by vehicle emissions and pollen dispersion, while the behavior of HULIS in summer was similar to photochemical oxidant and nitrogen dioxide concentrations. The proportion of aliphatic protons for summer samples was higher than that for spring samples, while the proportion of aromatic protons for summer samples was lower than that for spring samples. These seasonal changes of the chemical structure may also involve in their functional expression in the atmosphere.

Key words: Humic-like substances, Aerosols, ¹H nuclear magnetic resonance, ¹³C nuclear magnetic resonance

1. INTRODUCTION

Humic-like substances (HULIS) are the major components of water soluble organic carbon (WSOC) and may account for 9-72% of the WSOC in aerosols (Zheng *et al.*, 2013). HULIS play important roles in atmospheric processes because of their strong surface activity and light-absorbing capacity, which respond to cloud condensation nuclei and ice nuclei (Wang and Knopf, 2011; Sun and Ariya, 2006) and to brown carbon (Hoffer *et al.*, 2006), respectively. Identifying the chemical structural properties of HULIS is necessary to understand their function in the atmospheric environment, since the strength of the functions appears to depend on their chemical structure. Nevertheless, structural analysis of HULIS is challenging because of their complexity and heterogeneity.

Several analytical approaches such as spectroscopic techniques (UV-vis, Fourier transform infrared (FTIR), fluorescence, nuclear magnetic resonance (NMR)) (Havers *et al.*, 1998), size exclusion chromatography (Krivácsy *et al.*, 2001), elemental analysis (Kiss *et al.*, 2002), isotope ratio mass spectrometry (Song *et al.*, 2012), high performance liquid chromatography-electrospray ionization mass spectrometry (Kiss *et al.*, 2003), liquid chromatography tandem mass spectrometry (Stone *et al.*, 2009), pyrolysis gas chromatography/mass spectrometry (Fan *et al.*, 2013), Fourier transform ion cyclotron resonance ultrahigh resolution mass spectrometry (Lin *et al.*, 2012), and X-ray photoelectron spectroscopy (Song *et al.*, 2012) have been used to reveal the characteristics of HULIS. The chemical features of HULIS include high-molecular weight (MW), water-soluble, and oxygenated functional groups like carboxylic acids, yet the formation mechanisms of HULIS are poorly understood and may

include both primary and secondary sources (Graber and Rudich, 2006). Several studies have suggested that HULIS are a product of biomass burning (Feczko *et al.*, 2007; Hoffer *et al.*, 2006) and/or secondary processing via the transformation of gas and condensed-phase species by chemical reactions (Graber and Rudich, 2006; Gelencser *et al.*, 2002). A better understanding of the chemical structure of HULIS may provide insight into the formation pathways.

^1H and ^{13}C NMR are regarded as essential methods to evaluate the functional groups and overall chemical structure of natural organic matter (Hedges *et al.*, 2000). In atmospheric chemistry research, ^1H NMR spectroscopy has been widely used for determining the functional groups of HULIS and for revealing the source apportionment of aerosols, and valuable scientific knowledge has been accumulated with this technique (Chalbot *et al.*, 2014). For instance, Havers *et al.* (1998) reported that the ^1H NMR data on HULIS isolated from NIST (National Institute of Standards and Technology) 1648 urban particulate matter showed strong resonance signals in the aliphatic (0.7-1.9 ppm; ~50%) and carbohydrate (3.3-4.5 ppm; 40%) regions and, to a lesser extent, resonance signals in the aromatic region (6.7-8.3 ppm; 10%). Analyses of the NMR signals of HULIS have shown that the carbohydrate content is dependent on the contribution of compounds generated by biomass burning, while the aromatics arise from anthropogenic sources (Fan *et al.*, 2013). Furthermore, using ^1H -NMR and isotope ratio mass spectrometry, Chalbot *et al.* (2016) demonstrated that biomass burning and secondary aerosol dominated water-soluble organic matter in the summer, and pollen particles accounted for most of water-soluble organic matter in spring.

On the other hand, application of ^{13}C NMR spectroscopy for atmospheric samples is still limited because of the low sensitivity of such data. Although a few reports of solid state ^{13}C NMR spectra for HULIS with relatively low resolution exist (Duarte *et al.*, 2015, 2007, 2005; Sannigrahi *et al.*, 2006), quantitative reports about liquid-state ^{13}C NMR spectra for HULIS with relatively high resolution are lacking. The greatest benefit of ^{13}C NMR applications is that information on the skeletal C of HULIS can be obtained directly, and this technique represents a suitable tool for investigating the distribution of C functional groups; it may even be preferable to ^1H NMR for this purpose. Other benefits include the wide spread of ^{13}C chemical shifts, the reduced effects of salts, and the absence of large solvent resonance signals (Chalbot and Kavouras, 2014). However, ^{13}C NMR has low sensitivity (^{13}C NMR is approximately eight times lower than that of ^1H NMR) because the natural abundance of ^{13}C is no more than 1.1%, and thus, this technique requires large amounts

of sample material for measurements. This represents a significant limitation during ^{13}C detection for atmospheric applications (Chalbot and Kavouras, 2014).

Several methods including solid phase extraction (SPE), ion exchange chromatography, and size exclusion chromatography as well as their combination have been applied during the isolation of HULIS (Badael *et al.*, 2009; Samburova *et al.*, 2007; Decesari *et al.*, 2000; Krivacsy *et al.*, 2000). Among these methods, SPE is commonly used for isolation of HULIS from other dissolved constituents because of the easy implementation protocol and selectivity of the technique. For the isolation of HULIS using the SPE method, hydrophilic-lipophilic balance (HLB), C18, DAX-8 (or XAD-8), and diethylaminoethyl (DEAE) resins have been used (Fan *et al.*, 2012; Okochi *et al.*, 2008; Duarte and Duarte, 2005; Varga *et al.*, 2001). The HLB, C18, and DAX-8 resins isolate HULIS based on hydrophobic-hydrophilic interactions, whereas DEAE separates HULIS through an anion exchange process. It is assumed that these different methods isolate HULIS with different chemical properties depending on the specific properties of each resin. Indeed, Fan *et al.* (2012) reported that the chemical properties of HULIS prepared by four types of resins (HLB, C18, DAX-8, and DEAE) were different from each other.

The objectives of this study were as follows: 1) to investigate the influence of isolation methods on HULIS chemical structure data with special reference to liquid-state ^1H and ^{13}C NMR spectra; 2) to clarify the relationship between the behavior of HULIS in atmospheric aerosols and the chemical structure in samples from central Tokyo. To achieve our aims, HULIS in urban atmospheric aerosols were isolated by DEAE and HLB resins, which are widely used as separation media for HULIS, and then, we characterized the liquid-state ^1H and ^{13}C NMR spectra. Moreover, we estimated HULIS concentrations at 12 h intervals during the spring and summer.

2. MATERIALS AND METHODS

2.1 Sampling Site

Aerosol samples were collected on the rooftop of a building (ca. 65 m) that is part of the Nishi-Waseda campus (35.7°N, 139.7°E) of Waseda University, which is located in the Shinjuku ward of Tokyo, Japan. Shinjuku is a central region of Tokyo and over 300,000 people live there. The area around Shinjuku station, which is situated at the center of Shinjuku, consists of numerous businesses, commercial enterprises, and entertainment facilities. The sampling site is located along Meiji Street, which typically receives 36,000 cars/day, and

it is about 2 km north of Shinjuku station, which is located at about 6 km in the northwest direction away from Tokyo Bay.

In this study, we refer to the concentrations of several atmospheric pollutants (SO_2 , NO_2 , and O_3), and these data were measured at the Shinjuku city office monitoring station, which is located 1.5 km away from the urban study site. The weather conditions (temperature, relative humidity, rainfall, wind direction, and wind velocity) were monitored from the Tokyo District Meteorological Observatory, which is located 4.5 km away from the urban study site.

2.2 Isolation of HULIS by DEAE and HLB Resin

Suspended particulate matter (SPM) was collected on 8 inch \times 10 inch quartz fiber filter (Pallflex 2500QAT-UP, PALL, Port Washington, USA) with a high-volume sampler (HV-1000F, SHIBATA, Saitama, Japan) equipped with an impactor for SPM (100% cut-off at 10 μm , SHIBATA, Saitama, Japan) and set to a flow rate of 1000 L/min. This sampling took place during a 1 week period, 3-29 March 2016; the mean air temperature was 10.1°C, precipitation was 103 mm, and the mean relative humidity was 61%. Before sampling the aerosols, quartz filters were heated in a furnace for 4 h at 450°C to remove all organic contamination. Sampled filters were then stored at -18°C in a freezer. It should be noted that quartz fiber filters may adsorb gas-phase compounds, and semi-volatile compounds collected on the filter may evaporate over time. However, it was difficult to quantify these effects for the ambient conditions in the current study.

The SPM collected on the quartz fiber filter was placed in glass conical flask and 500 mL of Milli-Q water (18 $\text{M}\Omega\cdot\text{cm}$) was added. The WSOC was extracted by ultrasonication for 1 h after shaking the samples manually for 1 min. After ultrasonic extraction, the suspension was filtered through a 0.5 μm glass fiber filter (GC50, ADVANTEC, Tokyo, Japan), and then, the residue was washed with 500 mL Milli-Q water, filtrated again through a 0.5 μm glass fiber filter, and combined with the extract from the previous step. The above procedures were performed twice for each filter. To obtain sufficient mass for ^{13}C NMR measurements, the aqueous extracts were batched together according to the season (spring or summer) before the isolation of HULIS. The extracts were stored at 5°C in the dark.

Isolation of HULIS by DEAE resin was based on a method described in the previous literature (Havers *et al.*, 1998). A glass column (25 mm inner diameter \times 100 mm length, Bio-Rad, Hercules, California, USA) containing DEAE resins (TOYOPEARL 650M, parti-

cle size 65 μm , pore size 100 nm, TOSOH, Tokyo, Japan) was set up for the isolation of HULIS. This resin is composed of a base material of hydroxylated methacrylic polymer beads, which have been functionalized with DEAE weak anion exchange groups. The adsorption property of this resin may be slightly different from that of DEAE cellulose such as Whatman DE52 (GE Healthcare, Buckinghamshire, England, UK) and Wako DEAE Cellulose (Wako, Osaka, Japan), which was used for the determination of HULIS concentrations in urban atmospheric aerosol particles described in Section 3.2. Prior to usage, resins were rinsed with 2 L of 1 M NaCl, 0.1 M HCl, and 0.1 M NaOH, and finally, the resins were washed with 2 L Milli-Q water at a flow rate of 5 mL/min. Half of the aerosol extracts were passed through the glass column containing DEAE resins without any pre-treatment at a flow rate of 3 mL/min. As described below, another half of the aerosol extracts was used for HLB SPE cartridge. The column was rinsed with 1 L Milli-Q water to remove matrix components. HULIS retained on the column was extracted with 0.1 M NaOH, converted to the hydrogen form (H-form) by passing through an H^+ -type cation exchanger (DOWEX 50WX2, particle size 100-200 μm , Dow, Midland, Michigan, USA), and concentrated to 20 mL by using rotary evaporator at 35°C. The H^+ -form HULIS solution was neutralized to pH 7.0 by 0.1 M NaOH, and powdered HULIS samples were then obtained by freeze-drying ($\text{HULIS}_{\text{DEAE}}$).

At first, the HLB SPE cartridge (Oasis HLB, particle size 30 μm , pore size 8 nm, specific surface area 800 m^2/g , 500 mg/cartridge, Waters, Milford, Massachusetts, USA) was activated with methanol and Milli-Q water, and the pH values of the prepared aqueous aerosol extracts were adjusted to 2 by using 1 M HCl. Then, the solute was spiked into the HLB SPE cartridge. The sorbent was rinsed with 50 mL Milli-Q water to remove other constituents not retained on the HLB SPE cartridge. After this step, the sorbent column was carried off for drying under a stream of N_2 . The HULIS retained on the HLB column was eluted with 2% (v/v) ammonia/methanol (Lin *et al.*, 2010). The resulting eluate was evaporated to dryness under a stream of N_2 , and then, it was re-dissolved in 10 mL Milli-Q water. Finally, powdered HULIS samples isolated by the HLB cartridge were obtained by freeze-drying ($\text{HULIS}_{\text{HLB}}$).

To check for laboratory contamination, we ran blanks using blank filters for each method through the above procedures. The organic carbon in the blanks was negligible for our methods.

2.3 ^1H and ^{13}C NMR

The liquid-state ^1H and ^{13}C NMR spectra were re-

corded on a Bruker AVANCE 600 spectrometer (Bruker GmbH, Karlsruhe, Germany) operating at 600 MHz for ^1H and at 150 MHz for ^{13}C . Each sample (about 30 mg) was dissolved in 0.6 mL of 0.5 mol/L NaOD in D_2O within a 5 mm diameter sample tube. The chemical shifts were referenced to sodium 3-(trimethylsilyl) propionate-2,2,3,3- d_4 (0 ppm).

The ^1H NMR spectra were obtained by the homo-gate decoupling technique under the following conditions: pulse width, 6.5 μs ; acquisition time, 3.91 s; pulse delay, 2.00 s. The HOD proton (4.8 ppm) of water impurities was irradiated, and 16 scans were accumulated. Chemical shift assignments were referred to as presented in Chalbot *et al.* (2014). The resonance areas were calculated by means of electronic integration. The spectra were divided into the following four regions (Chalbot and Kavouras, 2014): (1) 0.7-1.9 ppm (H-C), which includes protons from methyl (R- CH_3), methylene (R- CH_2), and methyne (R-CH) groups; (2) 1.9-3.2 ppm (H-C-C=), which includes protons bound to the carbon in the α -position adjacent to a double bond in allylic (H-C $_{\alpha}$ -C=C) and carbonyl or imino (H-C $_{\alpha}$ -C=O or H-C $_{\alpha}$ -C=N, respectively) groups and protons in secondary and tertiary amines (H-C=NR $_2$ and NR $_3$, respectively); (3) 3.3-4.5 ppm (H-C-O), which includes protons bound to oxygenated saturated aliphatic carbon atoms in alcohols, polyols, ethers, esters, and organic nitrate (R- CH_2 -O-NO $_2$); (4) 6.7-8.3 ppm (Ar-H), which includes protons bound to aromatic carbon. A carbonylic/carboxylic aliphatic group (H-C-C=O) was estimated by subtracting the estimated aromatic contribution from H-C-C= (Decesari *et al.*, 2007).

The ^{13}C NMR spectra were quantified by proton decoupling of the ^{13}C signals by means of the inverse-gated decoupling technique under the following operating conditions: pulse width, 6.5 μs ; acquisition time, 0.54 s; pulse delay, 2.50 s. A total of 15,360 scans were accumulated, and line broadening was set at 50 Hz. The peaks in the ^{13}C NMR spectra of the samples were assigned according to the reports of Malcolm (1990) and Kögel-Knabner (2002). The resonance areas were calculated by means of electronic integration. The spectra were divided into the following five regions: alkyl C, 0-48 ppm; alkyl C substituted with O or N (O-alkyl C), 48-110 ppm; aromatic C, 110-165 ppm; and carboxyl C, 165-190 ppm (Fujitake and Kawahigashi, 1999).

As a reference sample, we obtained Lake Biwa fulvic acids (LBFA) from the Japanese Humic Substances Society. Their sampling site, isolation methods, and chemical properties (elemental composition, weight-average molecular weight, fluorescence spectrum, ^1H and ^{13}C NMR spectra) are described in Fujitake *et al.* (2009).

2.4 Estimation of HULIS Concentrations in the Urban Atmosphere

Sampling campaigns were conducted from 6 to 11 April 2015 (spring season) and from 12 to 17 July 2016 (summer season). The aerosol samples were collected by using a high volume air sampler operating at a flow rate of 1000 L/min over the course of 12 h from 06:00 JST to 18:00 JST (JST: Japanese Standard Time = UTC + 9 h). Particles smaller than 10 μm were collected onto a quartz fiber filter with a cascade impactor to avoid collecting large particles. Before sampling of the aerosols, quartz filters were heated in a furnace for 4 h at 450°C to remove all organic contaminants. Sample filters were then stored at -18°C in a freezer.

Determinations of HULIS concentrations were performed according to the method of Yamanokoshi *et al.* (2014). Briefly, portions of quartz filters (21 cm^2) were put into 50 mL glass centrifuge tubes and 50 mL of Milli-Q water was added. The tubes were continuously shaken for 1 h. After shaking, the suspension was centrifuged at 2000 g for 10 min and the supernatant was filtered through a 0.5 μm glass fiber filter; then, the residue was washed with 50 mL Milli-Q water. The washings were obtained by centrifugation and filtration and combined with the extract.

The DEAE method has been shown to have certain advantages such as enabling direct isolation without the use of any pre-acidification step. Therefore, we used DEAE resin to estimate the HULIS concentrations. HULIS were concentrated by sorption on a DEAE-cellulose (Cellufine MAX DEAE, JNC, Tokyo, Japan) column followed by elution with 5 mL of a 0.1 M sodium hydroxide solution via backward-flow procedures. The absorbance of the effluent (5 mL) was measured with a UV/VIS spectrophotometer (V-630, JASCO, Tokyo, Japan) at 400 nm (E_1). Because DEAE cellulose can collect negatively charged species other than HULIS, a correction was made as follows. Another aliquot of the filtrate, which was acidified to pH 1 with 4 M hydrochloric acid, was passed through a XAD-2 column. Humic substances are selectively and quantitatively sorbed on the XAD-2 in this process. After that, the column effluent was neutralized and introduced onto a DEAE-cellulose column. The sorbed negatively charged species were eluted and treated as described above. The absorbance of the effluent (5 mL) was also measured at 400 nm (E_2). The humic acid fraction (HA, acid-insoluble fraction in HULIS) and fulvic acid fraction (FA, acid-soluble fraction in HULIS) were fractionated at pH 1 by filtration, where a 0.45 μm Millipore membrane filter (25 mm in diameter) was first coated with sodium dodecyl sulfate (SDS-MF). The FAs pass nearly completely through the SDS-MF, while more than 90% of the HAs are retained on the SDS-MF

during this process (Hiraide *et al.*, 1994). The filtrate was adjusted to pH 13 with 0.1 M sodium hydroxide solution, and the absorbance of the filtrate (5 mL) was measured at 400 nm (E_3). The absorbance of HULIS (E_{HULIS}) was calculated by $E_1 - E_2$, the absorbance of FAs (E_{fulvic}) by $E_3 - E_2$, and the absorbance of HAs (E_{humic}) by $E_{\text{HULIS}} - E_{\text{fulvic}}$. We estimated the concentrations of HA, FA, and HULIS in WSOC by using Lambert-Beer's law. In this study, the absorbance coefficient of FA, which was extracted from brown forest soil, and that of HA, which was extracted from peat soil, at 400 nm were $3.8 \text{ mg}^{-1} \text{ cm}^2$ and $12 \text{ mg}^{-1} \text{ cm}^2$ at pH 13, respectively. There are no available data on the absorptivity of atmospheric HULIS; therefore, we applied these values to calculate the concentrations of FA and HA (Yamanokoshi *et al.*, 2014; Okochi *et al.*, 2008). The concentration of HULIS is the sum of the concentrations of HA and FA.

To examine the seasonal changes in chemical structure, we measured ^1H NMR spectra for HULIS isolated from summer samples. Summer samples were collected by using a high volume air sampler operating at 1000 L/min from 1 July to 31 August 2016 (mean air temperature, 26°C ; precipitation, 496 mm; mean rela-

tive humidity, 79%). HULIS were isolated by DEAE resin, and the ^1H NMR spectra were measured as described above.

3. RESULTS AND DISCUSSION

3.1 Characterization of HULIS Isolated by DEAE and HLB Resin

3.1.1 ^1H NMR Spectra

Fig. 1 shows the ^1H NMR spectra of HULIS and LBFA. The spectral shapes of $\text{HULIS}_{\text{HLB}}$ were similar to those of $\text{HULIS}_{\text{DEAE}}$. A few sharp signals were detected in the ^1H NMR spectra of HULIS (at around 0.86, 1.32, 1.92, 2.18, 2.41, 3.36, 3.95, 7.88, and 8.46 ppm), which may indicate that low molecular weight acids (e.g., acetate, 1.92 ppm; succinate, 2.41 ppm; formate, 8.47 ppm) associated with both primary and secondary organic aerosols were incorporated into and/or coexisted with the HULIS in the samples. Furthermore, $\text{HULIS}_{\text{HLB}}$ and $\text{HULIS}_{\text{DEAE}}$ showed a number of sharp peaks between 3.5 to 4.0 ppm and between 7.3 to 8.0 ppm, respectively (Fig. 1). These results suggest that

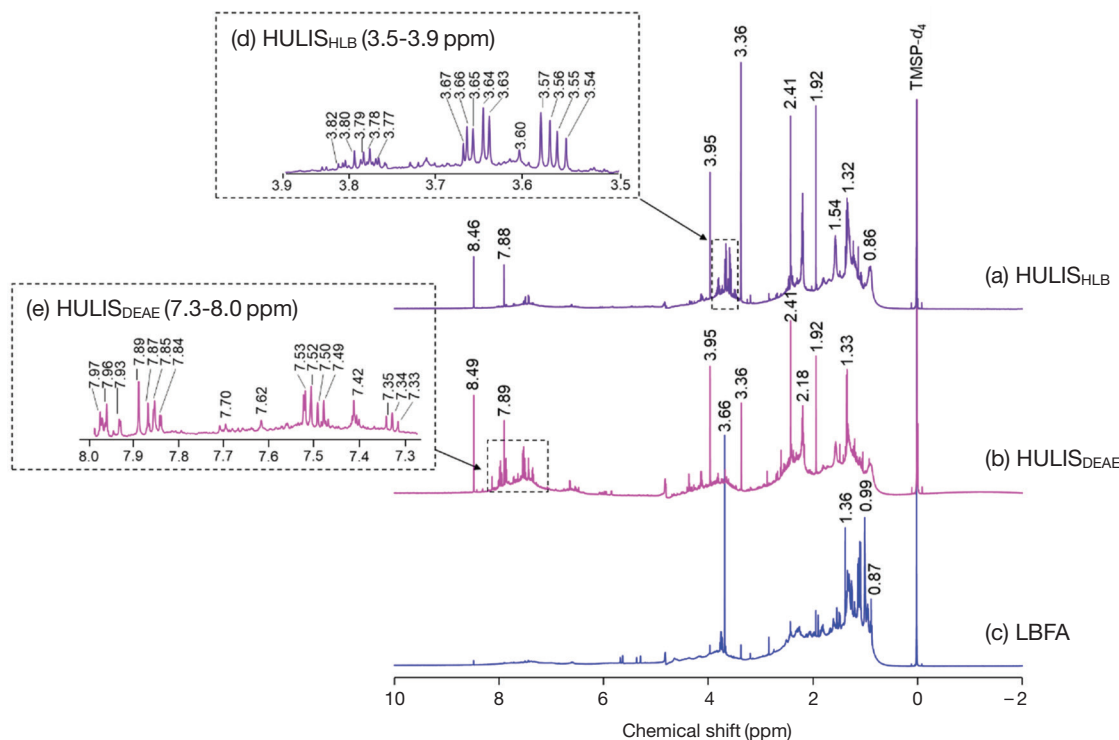


Fig. 1. ^1H NMR spectra of humic-like substances (HULIS) in urban atmospheric aerosols and fulvic acids isolated from a lake. (a) HULIS obtained from the spring sample by using HLB resin ($\text{HULIS}_{\text{HLB}}$), (b) HULIS obtained from the spring sample by using DEAE resin ($\text{HULIS}_{\text{DEAE}}$), (c) Lake Biwa fulvic acids (LBFA), (d) expanded spectrum of $\text{HULIS}_{\text{HLB}}$ (from 3.5 to 3.9 ppm), (e) expanded spectrum of $\text{HULIS}_{\text{DEAE}}$ (from 7.3 to 8.0 ppm).

low molecular weight sugars (e.g., glucose, 3.24, 3.37-3.43, 3.44-3.49, 3.52, 3.68-3.73, 3.74-3.77, 3.88-3.91, 3.81-3.85, and 5.23 ppm; fructose, 3.46, 3.55, 3.67, 3.75, 3.82, 3.87, 3.89, 4.06, 4.22, and 5.41 ppm; sucrose, 3.55-3.61, 3.66-3.73, 3.79-3.84, 3.89-3.91, 3.99-4.04, and 4.11-4.12 ppm) and aromatic hydrocarbons (e.g., benzoic acid, 7.45 and 7.62) may also have been incorporated into the HULIS. Therefore, it proved difficult to completely isolate only HULIS from the WSOC by using DEAE and HLB resins. However, most of the signals appeared as a continuous unresolved distribution, which suggests that a complex mixture of substances similar to that reported in previous studies was present.

The relative contents of the various H groups in HULIS and LBFA determined by integrating over the standard chemical shift range are shown in Table 1. Our results were similar to Song *et al.* (2012) and Fan *et al.* (2013). The HULIS contained relatively low contents of H-C and relatively high contents of Ar-H in comparison to LBFA, and proton compositions of HULIS were different from those of FAs isolated from water. Compared with HULIS_{HLB}, HULIS_{DEAE} contained relatively high contents of Ar-H and H-C-C= and relatively low contents of H-C, while the H-C-O contents were similar between these two types of samples. These findings are consistent with the results of Baduel *et al.* (2009), Fan *et al.* (2012), and Fan *et al.* (2013).

According to the work of Kawahigashi *et al.* (1996), the chemical shift region between 0 and 3.0 ppm was assigned to aliphatic protons (H_{al}), and this region was divided into the following three subregions: protons on terminal methyl groups attached to saturated aliphatic protons (H_{γ} , 0.0-0.9 ppm), protons on methylene β attached to olefins or aromatic rings (H_{β} , 0.9-1.6 ppm), and protons on methyl and methylene α attached to aromatic carbons, carbonyl groups, ester groups, and olefins (H_{α} , 1.6-3.0 ppm). The proportion of H_{γ} , H_{β} , and H_{α} in HULIS ranged from 4 to 8%, from 38 to 46%, and from 48 to 58%, respectively. The proportion of H_{β} was smaller than that of H_{α} , which indicates that the mean alkyl chain length of HULIS was short. In the spring samples, the proportion of H_{β} of HULIS_{HLB} was higher than that of HULIS_{DEAE} (HULIS_{HLB}, 46%; HULIS_{DEAE}, 38%), which suggests that HULIS_{HLB} was characterized by relatively long methylene chains in comparison with HULIS_{DEAE}. However, these values were lower than that of LBFA (55%), which indicates that the HULIS contained a low content of long-chain alkanes rather than hydrosphere FAs.

3.1.2 ^{13}C NMR Spectra

Fig. 2 shows the ^{13}C NMR spectra of HULIS and LBFA along with the soil humic acid (SHA) spectrum

taken from the literature (Katsumi *et al.*, 2016). According to Katsumi *et al.* (2016), soil sample was collected from A1 horizon of Inceptisols at Ishikawa Forest Experiment Station, where vegetation is dominated by *Cryptomeria japonica*. The ^{13}C NMR spectra exhibited four major broad peaks in the regions of alkyl C, O-alkyl C, aromatic C, and carboxyl C. The major sharp peaks in the ^{13}C NMR spectra of HULIS, LBFA, and SHA appeared at around 16, 29, 32, 34, 41, 52, 58, 65, 75, 130, 150, and 180 ppm. The peaks at around 16 and 32 ppm were attributed to terminal methyl groups and methylene groups in alkyl chains. The peak at 58 ppm in the SHA spectrum was assigned to methoxyl groups. The peaks at 65 ppm were assigned to C-6 in polysaccharides, and the peaks at around 75 ppm were ascribed to the carbon in CHO groups, ring carbon in polysaccharides, or ether bonded aliphatic carbon. The peaks at 130 and 150 ppm were attributed to C-substituted and H-substituted aromatic C and phenolic C, respectively. The peaks at around 170-180 ppm were assigned to carboxyl groups. A number of sharp peaks in the alkyl C region and signals at 65 and 75 ppm in the O-alkyl C region were observed in the ^{13}C NMR spectrum

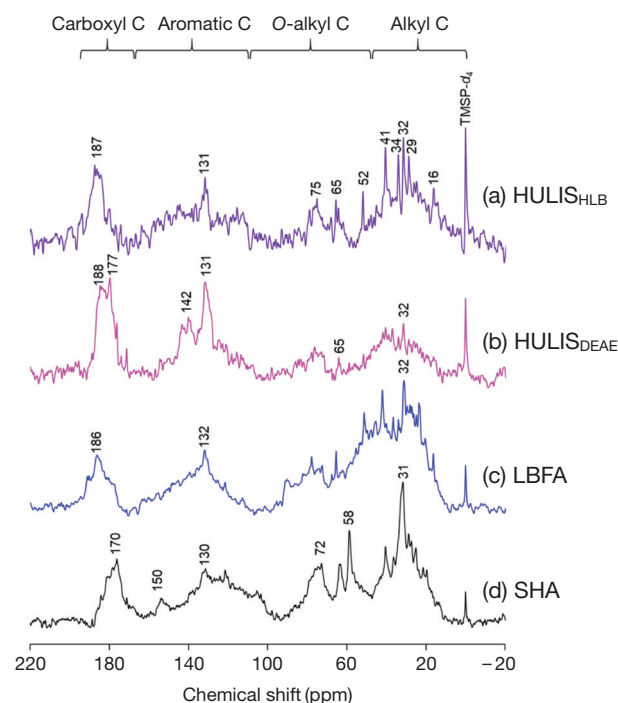


Fig. 2. ^{13}C NMR spectra of humic-like substances (HULIS), fulvic acids isolated from a lake, and soil humic acids (SHA). (a) HULIS obtained from the spring sample by using HLB resin (HULIS_{HLB}), (b) HULIS obtained from the spring sample by using DEAE resin (HULIS_{DEAE}), (c) Lake Biwa fulvic acids (LBFA), (d) SHA spectrum obtained from the literature [Katsumi *et al.*, 2016].

of HULIS_{HLB}, while these peaks displayed a continuous unresolved distribution in the ¹³C NMR spectrum of HULIS_{DEAE}. In the spectrum for SHA, methoxyl groups (58 ppm) and phenolic C (150 ppm) were clearly observed, which suggests that this material contained an altered lignin-like structure. Duarte *et al.* (2005) used solid-state ¹³C NMR to compare HULIS collected from an agricultural site in summer and late autumn. In their reports, the autumn sample showed resonances attributable to phenol and methoxyl groups, which are usually associated with lignin and its degradation products released during wood combustion. The signals for phenol and methoxyl groups were not detected in our HULIS samples, thus indicating that biomass burning contributed little to the source of HULIS in downtown Tokyo in spring. However, HULIS concentrations in autumn were positively correlated with levoglucosan, which is used as a chemical tracer for biomass burning, at our sampling site ($r=0.867$) (Yamanokoshi *et al.*, 2014); hence, biomass burning was a possible contributor to the HULIS in the autumn samples collected at our sampling site.

The relative contents of the various C functional groups determined by integrating over the standard chemical shift range are shown in Table 2. ¹³C NMR spectra of HULIS were characterized by relatively low *O*-alkyl C contents compared with SHA and LBFA. In comparison with LBFA, both HULIS_{DEAE} and HULIS_{HLB} were rich in aromatic C and carboxyl C and poor in alkyl and *O*-alkyl C, and these materials showed high aromaticity. However, Duarte *et al.* (2007) reported that HULIS in aerosols mostly consisted of alkyl C (40–62% of the total NMR peak area) followed by *O*-alkyl C (15–21%) and carboxyl C (5.4–13.4%); aromatic C was found to be a minor C species in that earlier study. These results are not in agreement with our results, and the discrepancy may have been due to the differences in sampling sites. Duarte *et al.* (2007) sampled PM_{2.5} in a rural area with high agricultural activity, while our sampling site was in downtown Tokyo, which is regarded as one of the largest cities in the world. According to Song *et al.* (2012), HULIS in urban atmospheric aerosols emitted from anthropogenic sources are rich in aromatic moieties. Therefore, the relative high content of aromatic C in our samples was likely the result of anthropogenic activities at our sampling site.

The proportion of alkyl C in HULIS_{HLB} was 1.5 times higher than that of HULIS_{DEAE}. On the other hand, the proportions of aromatic and carboxyl C in HULIS_{DEAE} were higher than those of HULIS_{HLB}. These results indicate that HULIS_{HLB} has aliphatic rich properties and HULIS_{DEAE} is rich in aromatic moieties; furthermore, the hydrophobic components were more selectively adsorbed to HLB resin, whereas DEAE resin selec-

tively retained aromatic carboxylic acids. These findings were consistent with the results of the ¹H NMR analysis. The above differences in the results may have been due to the fact that HLB resin is a hydrophilic-lipophilic-balanced reverse-phase sorbent and DEAE resin consists of weakly basic anion exchange resins.

The HULIS data showed high carboxyl C contents relative to LBFA and SHA. These carboxyl groups in HULIS may play a significant role in organic ligand reactions in the atmosphere. The acidic functional groups of HULIS can bind to a variety of metals ions (Okochi and Brimblecombe, 2002), and their chelating property may be particularly important for iron, a limiting nutrient for phytoplankton in the ocean (Paris and Desboeufs, 2013). On the other hand, Scheinhardt *et al.* (2013) suggested that complexation by HULIS is only significant for Fe³⁺ and Cu²⁺ and is generally less important than other ligands like oxalate and nitrate. Reports of HULIS complexation using actual atmospheric samples are limited, and further investigation of this issue will be necessary in the future.

3.2 Relationship between HULIS Behavior and the Chemical Structure

3.2.1 Diurnal Variation of HULIS Concentrations in Spring and Summer

Fig. 3 shows the diurnal variation of HULIS concentrations, weather conditions, and concentrations of atmospheric pollutants in central Tokyo during the spring campaign. HULIS were detected in all aerosol samples, and HULIS concentrations ranged from 0.388 to 0.819 $\mu\text{g}/\text{m}^3$ (mean $0.601 \pm 0.134 \mu\text{g}/\text{m}^3$). The concentrations of FA and HA ranged from 0.324 to 0.724 mg/L (mean $0.524 \pm 0.115 \mu\text{g}/\text{m}^3$) and from 0.012 to 0.178 $\mu\text{g}/\text{m}^3$ (mean $0.076 \pm 0.057 \mu\text{g}/\text{m}^3$), respectively. The FA were a major fraction in HULIS, and average ratios of FA to HULIS concentrations reached 87%. HULIS concentrations increased with increasing pollen dispersion during the daytime of 6 and 10 April. Although the behavior of HULIS concentrations in this spring sampling campaign did not show a clear relationship with the concentrations of atmospheric pollutants, Yamanokoshi *et al.* (2014) reported that HULIS concentrations at our sampling site in 2012 were positively correlated with SO₂, NO₂, and CO (SO₂, $r=0.697$; NO₂, $r=0.665$; CO, $r=0.729$; $n=19$). NO₂ emitted from vehicles and SO₂ emitted from ships account for 42.2% and 71.9% of the respective total NO₂ and SO₂ emissions in Tokyo (Environmental Bureau of the Tokyo Metropolitan Government, 2011), which suggests that NO₂ and SO₂ can be used as indicators of vehicle emissions and ship emissions, respectively. In addition, CO can be used as an indicator as incomplete combustion. Therefore,

Table 1. Distribution of proton species (%) for humic-like substances (HULIS).

Site	Site type	Sampling period	Season	Sampling type	Resin	H-C ^{a)}	H-C-C=O ^{b)}	H-C-C-O ^{c)}	Ar-H ^{d)}	Reference
Waseda University, Tokyo, Japan	Urban	Mar. 2016	Spring	SPM ^{e)}	DEAE	38	31	17	15	This study
Waseda University, Tokyo, Japan	Urban	Mar. 2016	Spring	SPM	HLB	49	25	19	7	This study
Waseda University, Tokyo, Japan	Urban	Jul.-Aug. 2016	Summer	SPM	DEAE	54	28	13	5	This study
Maofengshan Mountain Forest Park, Guangzhou, China	Coastal, suburban	Jul. 2006	Summer	TSP ^{f)}	HLB	54	27	16	4	Song <i>et al.</i> (2012)
Maofengshan Mountain Forest Park, Guangzhou, China	Coastal, suburban	Jan. 2007	Winter	TSP	HLB	60	21	10	9	Song <i>et al.</i> (2012)
University Town, Guangzhou, China	Coastal, suburban	Jul. 2006	Summer	TSP	HLB	56	26	11	7	Song <i>et al.</i> (2012)
University Town, Guangzhou, China	Coastal, suburban	Jan. 2007	Winter	TSP	HLB	55	23	13	9	Song <i>et al.</i> (2012)
Wushan, Guangzhou, China	Coastal, urban	Jul. 2006	Summer	TSP	HLB	58	20	13	9	Song <i>et al.</i> (2012)
Wushan, Guangzhou, China	Coastal, urban	Jan. 2007	Winter	TSP	HLB	54	24	12	9	Song <i>et al.</i> (2012)
Guangzhou, China	Urban	Aug.-Sep. 2011	Summer	PM _{2.5} ^{g)}	ENVI	46	33	15	6	Fan <i>et al.</i> (2013)
Guangzhou, China	Urban	Aug.-Sep. 2011	Summer	PM _{2.5}	HLB-M	41	35	18	6	Fan <i>et al.</i> (2013)
Guangzhou, China	Urban	Aug.-Sep. 2011	Summer	PM _{2.5}	HLB	47	33	13	7	Fan <i>et al.</i> (2013)
Guangzhou, China	Urban	Aug.-Sep. 2011	Summer	PM _{2.5}	XAD-8	44	37	13	6	Fan <i>et al.</i> (2013)
Guangzhou, China	Urban	Aug.-Sep. 2011	Summer	PM _{2.5}	DEAE	37	35	16	12	Fan <i>et al.</i> (2013)
Lake Biwa, Shiga, Japan	Clear lake	Oct.-Nov. 2001	Autumn	Lake water	DAX-8	66	22	11	2	This study

^{a)}H-C (0.5-1.9 ppm): H-C, methyl (R-CH₃), methylene (R-CH₂), and methyne (R-CH) groups; ^{b)}H-C-C = (1.9-3.2 ppm): includes protons bound to the carbon in the α -position adjacent to a double bond in allylic (H-C α -C=O) and carbonyl or imino (H-C α -C=N, respectively) groups and protons in secondary and tertiary amines (H-C=NR₂ and NR₃, respectively); ^{c)}H-C-O (3.3-4.5 ppm): includes protons bound to oxygenated saturated aliphatic carbon atoms in alcohols, polyols, ethers, esters, and organic nitrate (R-CH₂-O-NO₂); ^{d)}Ar-H (6.7-8.3 ppm): includes protons bound to aromatic carbon; ^{e)}TSP, total suspended particulate matter; ^{f)}SPM, suspended particulate matter; ^{g)}PM, particulate matter.

Table 2. Distribution of carbon species (%) and aromaticity for humic-like substances (HULIS).

Site	Site type	Sampling period	Season	Sampling type	Resin	C _{al} ^{a)}	C _{OMe} ^{b)}	C _{ar} ^{c)}	C _{COOH} ^{d)}	Aromaticity ^{e)}	Reference
Waseda University, Tokyo, Japan	Urban	Mar. 2016	Spring	SPM ^{f)}	DEAE	25	13	35	27	0.48	This study
Waseda University, Tokyo, Japan	Urban	Mar. 2016	Spring	SPM	HLB	36	17	29	18	0.35	This study
Aveiro, Portugal	Rural-coastal	Jul.-Aug. 2002	Summer	PM _{2.5} ^{g)}	XAD-8	62	21	6	11	0.07	Duarte <i>et al.</i> (2007)
Aveiro, Portugal	Rural-coastal	Oct.-Nov. 2002	Autumn	PM _{2.5}	XAD-8	61	26	7	7	0.07	Duarte <i>et al.</i> (2007)
Aveiro, Portugal	Rural-coastal	Nov.-Dec. 2002	Autumn	PM _{2.5}	XAD-8	50	24	16	11	0.18	Duarte <i>et al.</i> (2007)
Aveiro, Portugal	Rural-coastal	Dec. 2002	Winter	PM _{2.5}	XAD-8	49	27	14	10	0.16	Duarte <i>et al.</i> (2007)
Aveiro, Portugal	Rural-coastal	Jan. 2003	Winter	PM _{2.5}	XAD-8	41	36	15	8	0.17	Duarte <i>et al.</i> (2007)
Aveiro, Portugal	Rural-coastal	May-Jun. 2003	Summer	PM _{2.5}	XAD-8	52	23	10	15	0.12	Duarte <i>et al.</i> (2007)
Aveiro, Portugal	Rural-coastal	Jul. 2003	Summer	PM _{2.5}	XAD-8	52	23	12	13	0.14	Duarte <i>et al.</i> (2007)

^{a)}C_{Al}, alkyl C; ^{b)}C_{OMe}, O-alkyl C; ^{c)}C_{Ar}, aromatic C; ^{d)}C_{COOH}, carboxyl C; ^{e)}aromaticity: (aromatic C)/(alkyl C + O-alkyl C + aromatic C); ^{f)}SPM, suspended particulate matter; ^{g)}PM_{2.5}, particulate matter less than 2.5 microns in diameter.

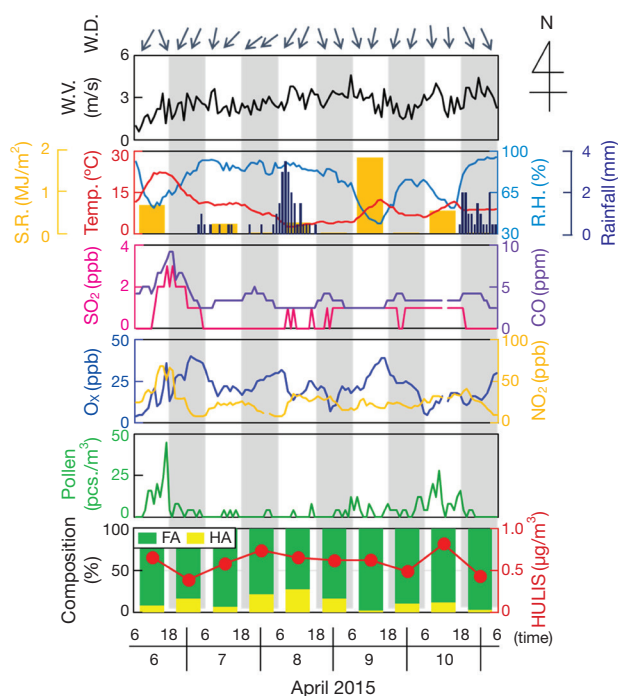


Fig. 3. Diurnal variation of humic-like substances (HULIS) concentrations and composition (fulvic acids, FA; humic acids, HA), weather conditions, and concentrations of atmospheric pollutants in central Tokyo during the spring sampling campaign. Concentrations of several atmospheric pollutants (SO_2 , NO_2 , and O_x) were measured at the Shinjuku city office monitoring station, which is located 1.5 km away from the urban study site. The weather conditions (solar radiation (S.R.), temperature (Temp.), relative humidity (R.H.), rainfall, wind direction (W.D.), and wind velocity (W.V.)) were monitored from the Tokyo District Meteorological Observatory, which is located 4.5 km away from the urban study site.

HULIS concentrations in spring may have been influenced by both pollen dispersion and anthropogenic sources.

Fig. 4 shows the diurnal variation of HULIS concentrations, weather conditions, and concentrations of atmospheric pollutants in central Tokyo during the summer campaign. During the summer sampling campaign, precipitation was observed on the nighttime of 14 July (amount of precipitation, 15.5 mm). Other than that event, the amount of solar radiation was high in the daytime, and high O_x concentrations were observed during the daytime of 14 July. HULIS concentrations ranged from 0.070 to 1.70 $\mu\text{g}/\text{m}^3$, and the mean HULIS concentration was $1.276 \pm 0.316 \mu\text{g}/\text{m}^3$. The concentrations of FA and HA ranged from 0.449 to 1.31 $\mu\text{g}/\text{m}^3$ (mean $0.909 \pm 0.272 \mu\text{g}/\text{m}^3$) and from 0.258 to 0.546 $\mu\text{g}/\text{m}^3$ (mean $0.367 \pm 0.103 \mu\text{g}/\text{m}^3$), respectively. In summer, FA were also a major fraction of HULIS, and the

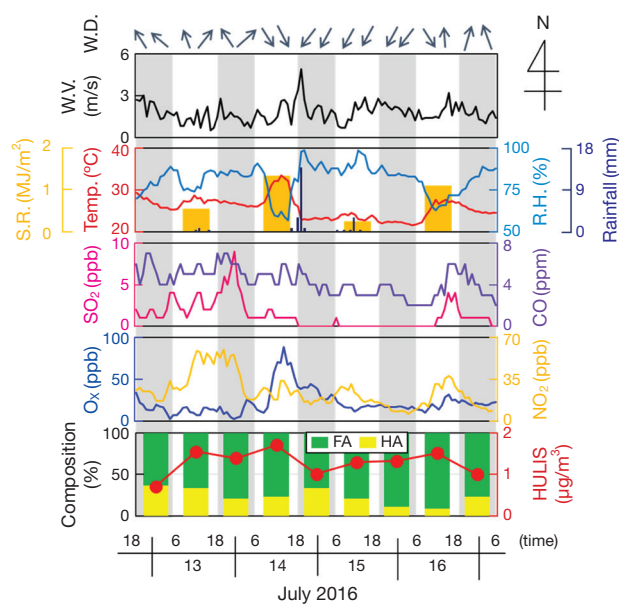


Fig. 4. Diurnal variation of humic-like substances (HULIS) concentrations and composition (fulvic acids, FA; humic acids, HA), weather conditions, and concentrations of atmospheric pollutants in central Tokyo during the summer sampling campaign. Concentrations of several atmospheric pollutants (SO_2 , NO_2 , and O_x) were measured at the Shinjuku city office monitoring station, which is located 1.5 km away from the urban study site. The weather conditions (solar radiation (S.R.), temperature (Temp.), relative humidity (R.H.), rainfall, wind direction (W.D.), and wind velocity (W.V.)) were monitored from the Tokyo District Meteorological Observatory, which is located 4.5 km away from the urban study site.

average ratio of FA to HULIS concentrations reached 71%. The behavior of HULIS concentrations in summer showed clear diurnal variation patterns similar to the O_x and NO_2 concentrations, i.e., increases in daytime and decreases in nighttime. HULIS concentrations were correlated with NO_2 ($r=0.909$) and O_x ($r=0.899$) during the summer sampling campaign. Yamanokoshi *et al.* (2014) also reported that HULIS concentrations were strongly correlated with NO_2 ($r=0.928$) and O_x ($r=0.750$) at our sampling site. O_x can be used as an indicator of the secondary formation of secondary organic aerosols (SOA) because O_x is generated from NO_x through photochemical reactions. These results suggest that HULIS are generated by secondary formation processes in addition to the primary sources in summer. Baduel *et al.* (2010) found that HULIS concentrations in urban atmospheric aerosols were highly and positively correlated with oxalic acid in summer, which suggests that SOA contribute to HULIS formation.

During the precipitation period of the sampling campaign, a decline in temperature and increase in relative

humidity were observed. As a result, atmospheric HULIS concentrations decreased. It seems likely that secondary formation did not occur because of the low amount of solar radiation and/or HULIS could have been scavenged by in-cloud and below-cloud scavenging processes.

3.2.2 Seasonal Changes of ^1H NMR Spectra

Seasonal changes in the chemical structure of HULIS were clearly observed between the spring and summer samples. The H-C content of the summer sample was 16% higher than that of the spring sample, while the Ar-H content was 10% lower than that of the spring sample. Therefore, HULIS influenced by secondary formation may be rich in H-C and poor in aromatics. These results were agreed with the previous report using ^{13}C NMR and FTIR (Duarte *et al.*, 2015). The increment of the H-C in summer indicates that the contribution of SOA increased, which is consistent with the results for the diurnal variation in the summer sampling campaign. On the other hand, the carbohydrate signature (H-C-O contents) of the spring samples was slightly higher than that of the summer samples because of the effect of pollen (Chalbot *et al.*, 2014).

4. CONCLUSIONS

In this study, we measured ^1H and ^{13}C NMR spectra of HULIS in urban atmospheric aerosols isolated by DEAE and HLB resin to characterize their chemical structure. HULIS_{DEAE} were characterized by relatively high contents of aromatic protons and relatively low contents of aliphatic protons in comparison with HULIS_{HLB}, while H-C-O proton contents were similar. The proportion of alkyl C in HULIS_{HLB} was higher than that of HULIS_{DEAE} according to the ^{13}C NMR spectra. On the other hand, the proportions of aromatic and carboxyl C in HULIS_{DEAE} were higher than those of HULIS_{HLB}. These results indicate that hydrophobic components are more selectively adsorbed onto the HLB resin and that DEAE resin selectively retains aromatic carboxylic acids. These points need to be considered when comparing the chemical structural properties of HULIS purified with different resins. In order to compare the chemical structural properties of HULIS in more detail, accumulation of these data and standardization of purification method are necessary.

The use of DEAE resin has certain advantages such as allowing for direct isolation without the use of any pre-acidification step. Moreover, DEAE resin has the highest selectivity for isolation of HULIS from atmospheric samples, compared with ENVI-18, HLB and XAD-8 (Fan *et al.*, 2012). On the other hand, the use of

HLB resin also has certain advantages, namely, there was a low level of inorganic impurities in the products due to the use of methanol as an eluent. However, the HLB method requires effort and a relatively long time period to the dry solvents. In contrast, the DEAE method is a rapid procedure for quantification of HULIS because the extracts can be introduced to the analysis equipment directly. A disadvantage of the DEAE method is that a high ionic strength solution is used as the eluent, which may cause potential interferences during the quantification and characterization of HULIS (Fan *et al.*, 2013). However, these inorganic salts can be removed by cation exchange resin or size exclusion chromatography. Therefore, DEAE resin seems to be well suited for the isolation of HULIS.

By combining atmospheric observations and NMR analyses, we demonstrated that the chemical structural features of HULIS are significantly different between summer and spring in reflection of their sources. The factors controlling the HULIS concentrations in atmospheric aerosols included vehicle emissions and pollen dispersion in spring based on the atmospheric environmental monitoring data and HULIS concentration data. On the other hand, the behavior of the HULIS was similar to O_x and NO_2 in summer. These results indicate that the sources of HULIS differed by season. Seasonal changes in the chemical structure of HULIS were clearly observed in the ^1H NMR spectra and were reflective of their sources. The H-C content of summer samples was higher than that of spring samples, while the Ar-H content of summer samples was lower than that of spring samples. These results suggest that HULIS originating from secondary formation processes may be rich in H-C content and poor in aromatics. On the other hand, the carbohydrate signature of spring samples was slightly higher than that of summer samples because of the presence of material of a biological origin (i.e., pollen). These seasonal changes in the chemical structure may involve in their functional expression in atmosphere. Further efforts will be required in the future to understand the linkage between their behavior, chemical structure, and functions in the atmosphere.

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