

Analysis of Natural Organic Matter in Water from Cold and Hot Mineral Springs in South Korea Using 15T FT-ICR-MS

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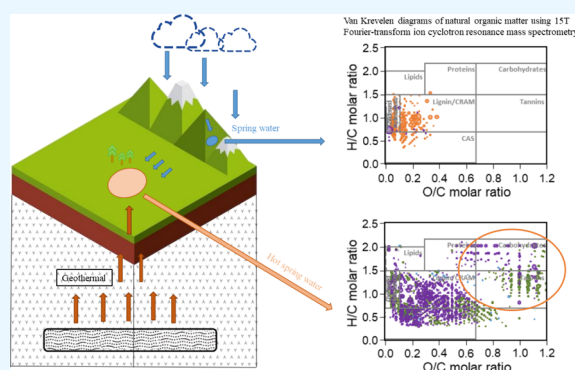
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ABSTRACT: The natural organic matter (NOM) properties in water from cold and hot mineral springs in South Korea are not well documented. We analyzed the characteristics of NOM in water from 25 cold and hot mineral springs located across South Korea. The NOM of each sample was concentrated using solid-phase extraction and analyzed using 15T Fourier-transform ion cyclotron resonance mass spectrometry. The origin of NOM was identified using van Krevelen diagrams. This study suggests that an analytical method to evaluate the characteristics of water in each region of South Korea can be established and used as a baseline for further research.



1. INTRODUCTION

Water is critical for humans and is an essential factor in supporting life.¹ For instance, Gleick² found that 50 L of water per person is necessary to avoid social and military conflicts. Moreover, the supply of clean water is essential. Natural organic matter (NOM) is found in accessible groundwater (cold mineral springs) and hot mineral springs. NOM produces carcinogenic byproducts, such as trihalomethanes and haloacetic acids, during chlorine disinfection in water treatment plants. Studies are ongoing on membrane separation technology for the treatment of NOM in water.^{3–5} However, NOM remains largely undefined,⁶ with a complex molecular composition, presenting an analytical challenge. We overcame this challenge using van Krevelen diagrams created using data obtained from Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Van Krevelen diagrams, which show the hydrogen-to-carbon molar ratio (H/C ratio) on the vertical axis and the oxygen-to-carbon molar ratio (O/C ratio) on the horizontal axis, can be used to differentiate the major biogeochemical classes of compounds (lignin, lipids, carbohydrates, etc.).⁷

FT-ICR-MS provides the up-to-date highest analytical resolution of dissolved organic matter (DOM) quality. However, only the elemental compositions of components can be analyzed and not the isomeric structures. FT-ICR-MS can identify complex organic mixtures using high-resolution and accurate MS without prior extraction or separation steps.⁸ FT-ICR-MS enables molecular-level analysis of complex mixtures.⁹ In addition, accurate mass measurements can be used to define the unique elemental composition from a single charged ion,

such as $[M + H]$, $[M + Na]$, $[M + K]$, and $[M + Cl]$.^{10–12} The advantage of FT-ICR-MS is its ability to study NOM at the molecular level through ultra-high-resolution and accurate mass measurement.

Using the available analytical technology over time, water research has identified various characteristics such as pH, calcium, chlorine, sodium, silicon, aluminum, magnesium, helium, neon, and zinc. Water analysis is important for evaluating potential positive or negative impacts for ecological risks, risk for health, and so forth.^{13–19} Recent developments in analytical technology have enabled the analysis of NOM that has not been previously studied. In addition, a study was conducted on the NOM analysis method of spring water.^{20–22} However, only few reports about NOM in springs exist; also, research on the NOM of cold and hot spring water in each region of South Korea is insufficient. In this study, we used ultra-sensitive 15T FT-ICR-MS to analyze and classify NOM in 25 samples collected from cold and hot mineral springs across South Korea.

We collected water from cold and hot mineral spring water sources in each region of South Korea for analysis of NOM. Cold spring water is used for drinking because it is clean in the natural state and can maintain water quality in groundwater or spring

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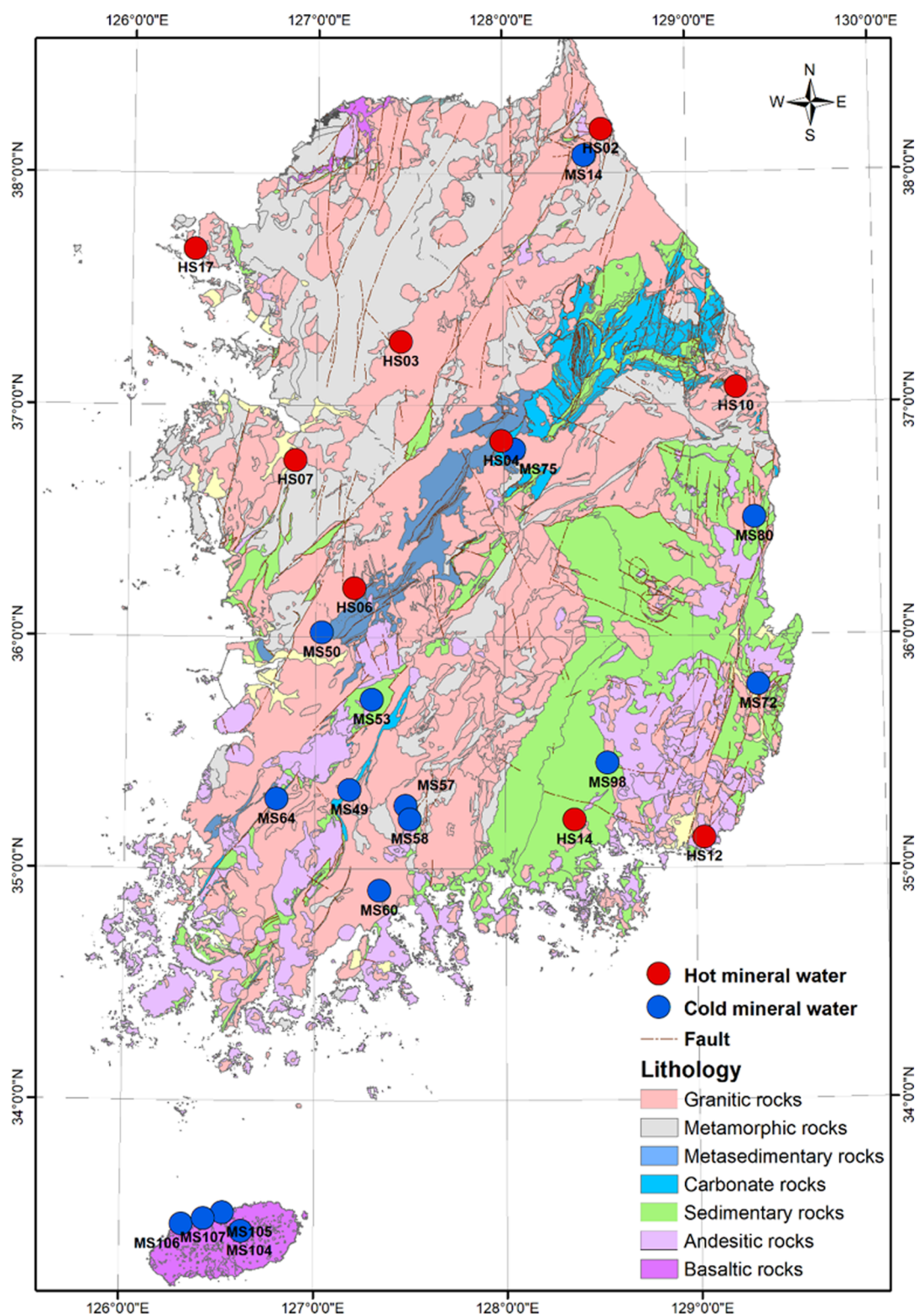


Figure 1. Map of South Korea showing geology and sampling sites.

water in bedrock aquifers. Given its mountainous terrain, South Korea has many sources of cold spring water, and the largest number of samples in this study came from such sources.²³ Hot spring water is defined differently depending on the criteria used. In South Korea, hot spring water is defined as water with a temperature of at least 25 °C, whose components are not harmful to the human body. According to the pH, it is classified as acid, neutral, or alkaline, and hot springs with a salt content of

less than 1 g/kg are called simple springs, which is the predominant type in South Korea.²⁴

2. MATERIALS AND METHODS

2.1. Site Description. Sampling was carried out at 25 locations across South Korea (Figure 1). South Korea is located in the mid-latitudes of the Northern Hemisphere and

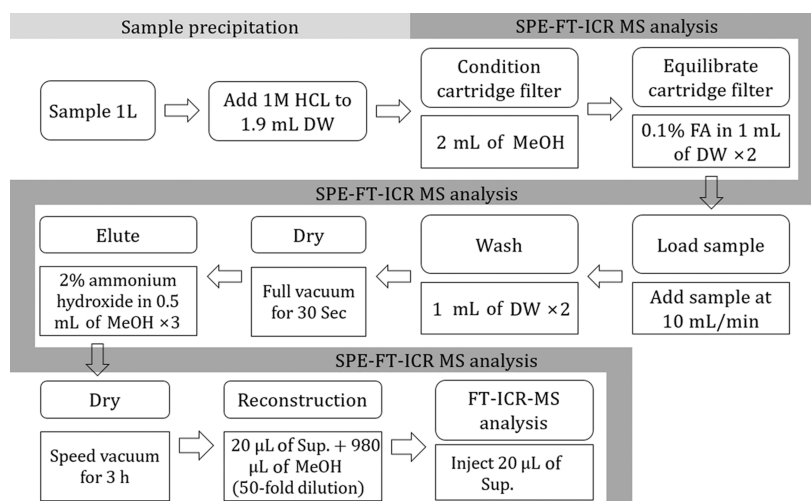


Figure 2. Schematic diagram of the SPE pre-processing step. DW: deionized water; FA: formic acid; FT-ICR-MS: Fourier-transform ion cyclotron resonance mass spectrometry; MeOH: methanol; and Sup: supernatant.

experiences distinct seasonal changes. Unlike the topographical features of the United States and Africa, South Korea consists primarily of mountainous terrain, with a lack of vast plains and an irregular river flow.²⁵ Due to seasonal wind, South Korea has high summer rainfall, and 44–57% of rainfall occurs during the summer rainy season.²⁶

The geology of South Korea is composed of metamorphic rocks, granitic rocks, and volcanic rocks, accounting for approximately 70% of the total area, and sedimentary rocks accounting for the remaining 30% (Figure 1). All hot mineral springs in South Korea are located in granite areas, and most of the cold mineral waters were collected from areas of granites and volcanic rocks (Figure 1). In South Korea, hot mineral spring water is groundwater with a temperature of 25 °C or higher (Lee, 2007).²⁷ In general, hot mineral springs are closely related to magma or volcanic activity, but the hot mineral springs in South Korea are non-volcanic hot mineral springs in which groundwater is heated by a geothermal gradient (Jeong et al., 2019).²⁸

Figure 1 shows the sampling points and the characteristics (type) of the water at each point. Hot mineral spring waters were collected from boreholes in nine hot springs in South Korea from August 2017 to March 2017. Cold mineral spring waters were collected from famous cold mineral springs used for drinking water in South Korea from September 2017 to October 2017. After sampling, the physicochemical properties of the samples (e.g., pH, electrical conductivity, temperature) were measured immediately at the site using an Orion 5-star portable meter. Water samples were collected using pre-washed high-density polyethylene bottles. Samples were placed on ice after collection and transported to the laboratory and then passed through a 0.45 μm cellulose membrane (diameter, 47 mm; Millipore, Burlington, MA, USA) using a vacuum filter. Filters were rinsed with Milli-Q water before filtration. The filtered samples were stored in a refrigerator (4 °C) and analyzed within 2 weeks of sampling.

2.2. Fluorescence Excitation–Emission Matrices. Fluorescence excitation–emission matrices (EEMs) were obtained with a luminescence spectrometer (F-7000, HITACHI, Japan). The EEMs were scanned at the emission wavelengths (Em) of 250–500 nm with 3 nm increments over an excitation wavelength (Ex) range between 250 and 500 nm with a stepwise

increase of 3 nm. The fluorescence responses to the distilled water were subtracted from the measured spectra to obtain the final EEM data of samples. The final fluorescence intensities were normalized to Raman units (R.U.) using the integrated Raman peak intensity at 350 nm (Em).²⁹

Three commonly used fluorescence indices were estimated from the EEM spectra, which included the fluorescence index (FI),³⁰ biological index (BIX),³¹ and humification index (HIX).³² The FI is the ratio of the emission intensity at 450 nm to that at 500 nm, obtained with an Ex of 370 nm. The index typically has a value of ~1.9 for DOM with relatively microbial origin and of ~1.4 for relatively terrestrial DOM sources.³⁰ The BIX is a rough descriptor for probing the presence of autochthonous DOM. The value is higher for samples enriched with freshly produced DOM from planktonic or microbial sources. It was calculated using a ratio of the integrated emission intensity from 380 to 430 nm at an Ex of 310 nm.³¹ The HIX has been utilized to indicate the degree of humification in samples, which was estimated with an emission intensity area from 435 to 480 nm divided by the sum of two intensity areas at 300–348 and 435–480 nm with an Ex of 254 nm.³² In this study, the peak-picking method was adopted to quantify different fluorescent components instead of parallel factor analysis due to the insufficient number of samples ($n = 26$). The excitation/emission wavelengths used for two protein-like and three humic-like peaks were 280/310 nm (B), 280/340 nm (T), 260/420 nm (A), 315/400 nm (M), and 350/450 nm (C), respectively, according to the definitions in a previous study.³³

2.3. Materials. We used high-performance liquid chromatography (HPLC)-grade formic acid and ammonium hydroxide purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (MeOH) was purchased from J.T. Baker (Phillipsburg, NJ, USA), and hydrochloric acid (HCl) was purchased from Samchun Chemicals (Pyeongtaek, South Korea). Ultrapure water was produced using a Milli-Q Integral system (Merck Millipore). Suwannee River fulvic acid was used as a standard for the characterization of NOM in samples.

2.4. Solid-Phase Extraction. Solid-phase extraction (SPE) was carried out with Bond Elut PPL 500 mg/6 mL cartridges (Agilent, Santa Clara, CA, USA). Sample pH was adjusted to pH 2.0 using HPLC-grade HCl (1 M). SPE cartridges were conditioned with 2 mL of MeOH and equilibrated with 2 mL

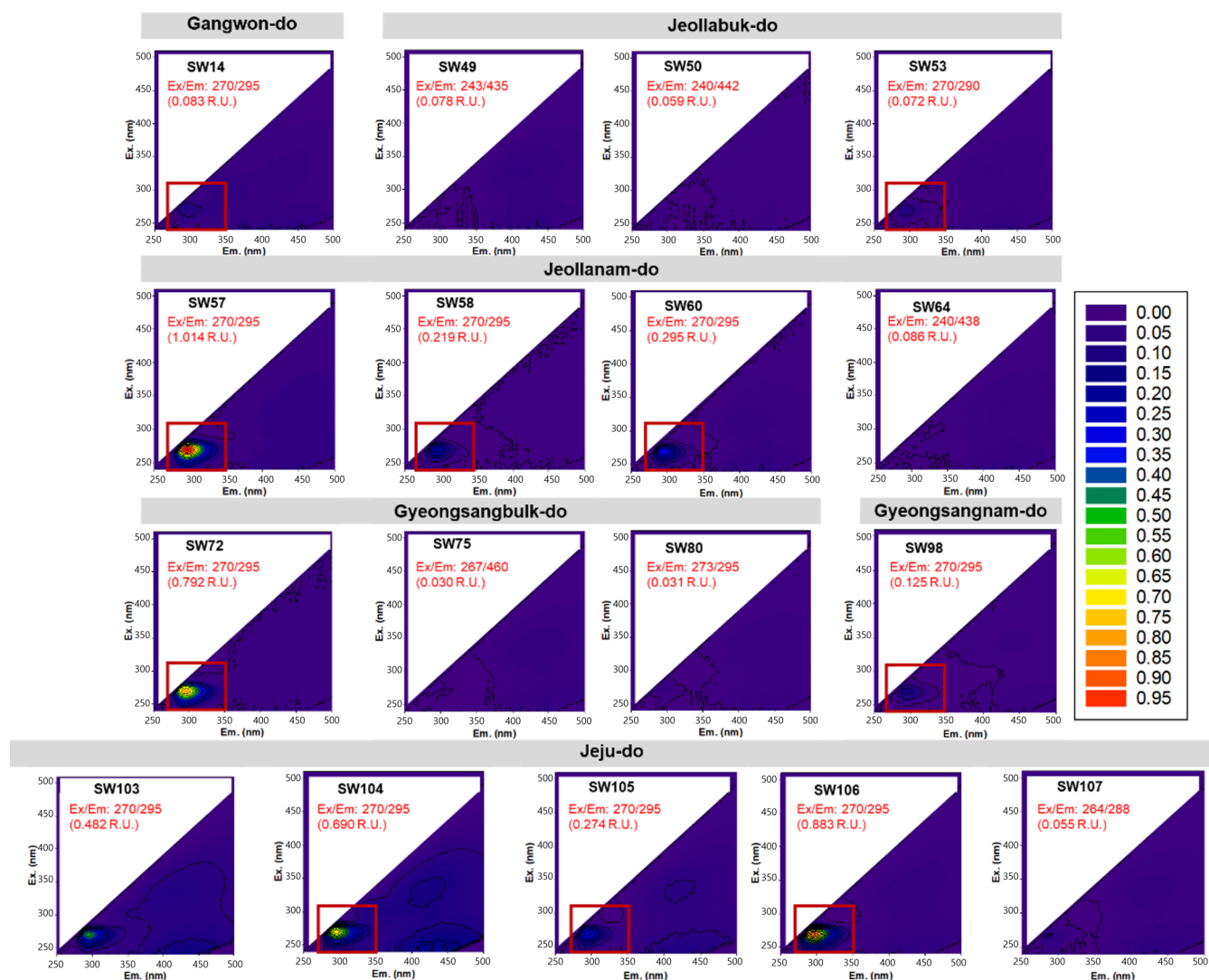


Figure 3. Fluorescence EEM spectra of cold spring water samples from different sampling sites in South Korea. The boxes and the letters in red refer to the positions of the strongest peak in the corresponding EEM and its intensity, respectively.

of 0.1% v/v formic acid in ultrapure water. Then, 1 L of the acidified sample was loaded into the cartridge at a flow rate of about 10 mL/min, washed with 2 mL of ultrapure water, and then eluted with 1.5 mL of 2% ammonium hydroxide in MeOH. The PPL cartridges were dried using pure nitrogen gas and stored at $-80\text{ }^{\circ}\text{C}$. Low concentrations of NOM were extracted from water samples using this method and analyzed using 15T FT-ICR-MS (15T-FT-ICR-MS, Bruker Daltonik, Germany). Figure 2 presents a schematic diagram of the extraction process.³⁴

2.5. Mass Spectrometry Analysis. The SPE samples were dissolved in 1 mL of MeOH and centrifuged at 13,000 rpm for 10 min. Samples were then diluted 50 times in 1 mL of MeOH and centrifuged at 13,000 rpm for 10 min. Analysis was carried out using 100 μL of the supernatant. 15 T FT-ICR-MS was interfaced with an Apollo II electrospray ionization source (Bruker Daltonics, Billerica, MA, USA), operating in the negative ion mode, and the instrument was operated in the broadband mode between 150 and 1200 m/z . The samples were injected using a 250 μL syringe (Hamilton, Reno, NV, USA) at a flow rate of 2 $\mu\text{L}/\text{min}$. Other MS parameters included a spray current of -3.0 kV and a dry gas temperature of $180\text{ }^{\circ}\text{C}$ with a

4.0 L/min skimmer voltage of -45 V . A total of 100 scans collected with a 4 MWord time domain were combined into one mass spectrum.

The results were classified into five components based on the molar O/C and H/C ratios: carbon, hydrogen, and oxygen compounds (CHO), carbon, hydrogen, oxygen, and nitrogen compounds (CHON), carbon, hydrogen, oxygen, and sulfur compounds (CHOS), carbon, hydrogen, oxygen, nitrogen, and sulfur compounds (CHONS), and Others. In addition, we analyzed eight molecular compound classes using van Krevelen diagrams:^{35,36} aromatic formulae, condensed aromatic structures (CASs), carbohydrates, lignin-/carboxyl-rich alicyclic molecules, lipids, proteins, unsaturated hydrocarbons (UnsatHydroC), and tannins.

The mass error tolerance was set as 1.0 ppm. For the formularity method, formulae of all peaks were assigned by following threshold values in the user-defined filter: $\text{O} > 0$, $\text{H}/\text{C} \leq 2.5$, $\text{O}/\text{C} \leq 1.2$, $\text{N} \leq 5$, $\text{S} \leq 3$, and $\text{P} \leq 2$.³⁶

3. RESULTS AND DISCUSSION

3.1. Fluorescence Analysis. A prominent peak appeared in the protein-like fluorescence region of the mineral water samples

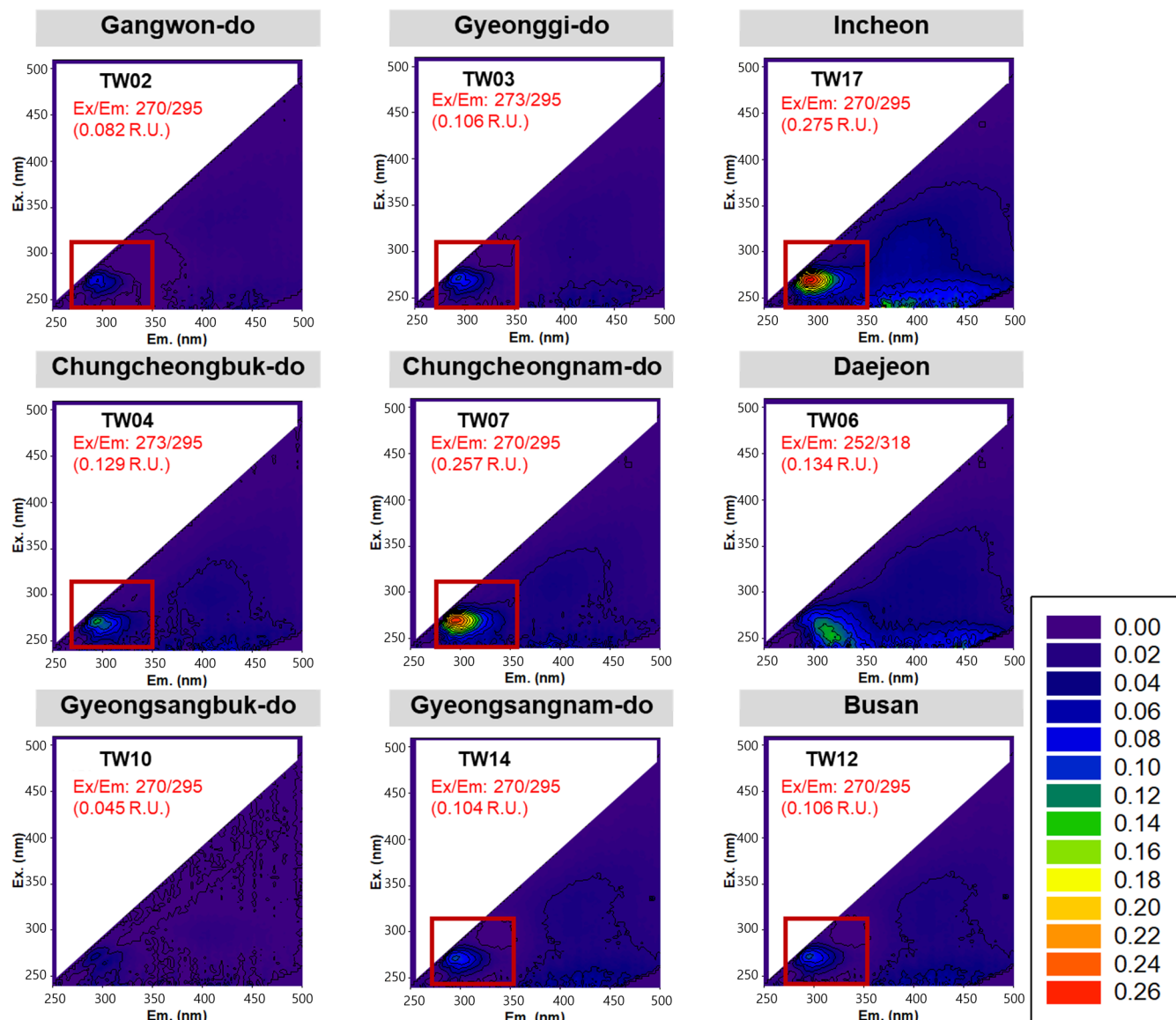


Figure 4. Fluorescence EEM spectra of hot spring water samples from different sampling sites in South Korea. The boxes and the letters in red refer to the positions of the strongest peak in the corresponding EEM and its intensity, respectively.

irrespective of the two different groups of the samples (i.e., hot vs cold springs), which was similar to peak B [$\sim 225(\sim 280)/\sim 305$ nm] defined by Coble (2007). For the entire studied area, the average intensities of peak P were 0.050 ± 0.038 R.U. for the hot spring and 0.064 ± 0.106 R.U. for the cold spring. For example, even among the cold water samples taken from the Jeolla region (i.e., SW49, 50, 53, 57, 58, 60, and 64), there were wide variations in the relative abundances that changed from 0.4 to 74.0%.

Insignificant difference was found in all the selected fluorescence peaks (i.e., peaks B, T, M, A, and C) between the two spring water groups (Student *t*-test, $p = 0.205$ for peak B, $p = 0.966$ for peak T, $p = 0.901$ for peak M, $p = 0.866$ for peak A, and $p = 0.274$ for peak C).

The commonly used fluorescence proxies, which have been widely used for describing different DOM sources, also revealed that no significant difference existed between the two spring water groups as shown by all the p values higher than 0.10 (HIX: 0.121, BIX: 0.418, FI: 0.452).

All taken together, it was found that fluorescence spectroscopy is limited to capturing any consistent differences in the chemical composition of spring water DOM arising from geological background.

The fluorescence EEM spectra of each sample are shown in Figures 3 and 4 and Tables 1 and 2.

3.2. Mass Spectrometry. **3.2.1. Cold Spring Water.** We analyzed a total of 16 water samples from cold springs; the results are shown in Table 3. Overall, the samples were dominated by CHON (60.17%), followed by CHONS (17.96%), CHOS (7.30%), and CHO (0.96%). Other substances were grouped as others (13.61%). The prevalence of CHON compounds may be due to high bacterial activity.³⁷ Nitrogen-containing components play an important role in stabilization due to the interaction of nitrogen with the mineral surface.³⁸ Nitrogen may also have been generated during the process of putrefaction of the soil in mountainous regions. In a study of springs near Lake Taihu, China, results were similar to those of the freshwater lake. NOM from these samples was mainly composed of CHO, followed by CHON, CHOS, and

Table 1. Summary of Fluorescence Properties for Cold Water Samples from Different Sampling Sites in South Korea

	Gangwon-do			Jeollabuk-do			Jeollanam-do			Gyeongsangbuk-do			Gyeongsangnam-do			Jeju-do		
	SW14	SW49	SW50	SW53	SW57	SW58	SW60	SW64	SW72	SW75	SW80	SW98	SW103	SW104	SW105	SW106	SW107	
FI (McKnight)	1.34	1.84	2.06	1.76	1.32	1.35	1.44	1.49	1.31	1.51	1.49	1.45	1.46	1.44	1.48	1.28	1.33	
BIX (Huguet)	1.43	0.81	0.59	0.74	0.49	0.34	0.69	0.64	0.49	0.39	0.44	0.21	0.85	0.95	0.74	0.67	0.67	
HIX (Ohno)	0.57	1.16	1.86	0.87	0.19	0.21	0.51	1.12	0.05	1.64	1.19	0.98	0.59	0.55	0.63	0.21	0.94	
B _{280/310}	0.029	0.005	0.000	0.018	0.330	0.045	0.064	0.005	0.236	0.008	0.002	0.026	0.158	0.238	0.078	0.259	0.009	
T _{280/340}	0.036	0.002	0.006	0.002	0.040	0.020	0.016	0.005	0.035	0.012	0.006	0.010	0.050	0.090	0.010	0.033	0.005	
M _{315/400}	0.026	0.029	0.006	0.017	0.022	0.005	0.022	0.017	0.005	0.021	0.013	0.004	0.074	0.102	0.046	0.022	0.019	
A _{260/420}	0.028	0.025	0.010	0.013	0.025	0.004	0.026	0.017	0.010	0.022	0.014	0.014	0.095	0.127	0.063	0.032	0.028	
C _{350/450}	0.019	0.023	0.007	0.016	0.028	0.006	0.016	0.018	0.003	0.024	0.017	0.011	0.070	0.095	0.045	0.024	0.019	
B _{280/310}	21.18	5.85	0.38	27.46	73.99	56.19	44.73	7.68	81.52	9.52	4.21	40.29	34.36	36.49	32.23	69.94	11.01	
T _{280/340}	25.87	2.88	19.81	6.55	9.07	24.59	11.02	7.97	12.14	14.05	11.36	15.01	9.01	13.83	4.12	8.93	6.51	
M _{315/400}	18.51	34.43	21.75	24.16	4.95	6.06	15.2	27.94	1.74	23.48	24.27	5.52	17.30	15.61	19.00	6.07	23.62	
A _{260/420}	20.05	29.33	34.89	19.11	5.72	5.27	18.13	27.45	3.58	25.41	27.34	22.04	22.67	19.43	25.91	8.60	34.24	
C _{350/450}	14.39	27.51	23.17	22.72	6.27	7.89	10.92	28.96	1.02	27.54	32.82	17.14	16.66	14.64	18.68	6.46	24.62	

Table 2. Summary of Fluorescence Properties for Hot Water Samples from Different Sampling Sites in South Korea

	Gangwon-do		Gyeonggi-do		Incheon		Chungcheongbuk-do		Chungcheongnam-do		Daejeon		Gyeonggangbuk-do		Gyeonggangnam-do		Busan	
	TW02	TW03	TW04	TW07	TW17	TW04	TW07	TW06	TW10	TW14	TW12							
FI (McKnight)	1.53	1.39	1.25	1.37	1.46	1.25	1.37	1.61	1.52	1.59	1.24							
BIX (Huguet)	0.39	0.58	0.77	1.09	1.06	0.77	1.09	0.87	0.24	0.79	0.82							
HIX (Ohno)	0.92	0.71	0.49	0.32	0.53	0.49	0.32	0.39	0.13	0.63	0.76							
B _{280/310}	0.012	0.03	0.042	0.103	0.114	0.042	0.103	0.072	0.007	0.028	0.047							
T _{280/340}	0.011	0.02	0.023	0.036	0.050	0.023	0.036	0.055	0.003	0.002	0.001							
M _{315/400}	0.007	0.02	0.022	0.028	0.049	0.022	0.028	0.034	0.004	0.045	0.023							
A _{260/420}	0.013	0.02	0.028	0.023	0.076	0.028	0.023	0.046	0.006	0.058	0.037							
C _{350/450}	0.011	0.01	0.015	0.017	0.030	0.015	0.017	0.025	0.000	0.036	0.019							
B _{280/310}	22.18	27.3	32.45	49.57	35.72	32.45	49.57	30.92	33.53	16.72	36.95							
T _{280/340}	20.59	18.9	17.23	17.54	15.62	17.23	17.54	23.65	13.29	1.11	0.61							
M _{315/400}	13.23	15.2	17.11	13.56	15.44	17.11	13.56	14.74	20.27	26.57	18.03							
A _{260/420}	23.97	22.8	21.69	11.11	23.91	21.69	11.11	19.78	31.43	34.51	29.34							
C _{350/450}	20.03	15.8	11.52	8.21	9.31	11.52	8.21	10.91	1.48	21.09	15.07							

Table 3. Molecular Characteristics of Cold Spring Water Samples from Different Sampling Sites in South Korea

sample	Gangwon-do			Jeollabuk-do			Jeollanam-do			Gyeongsangbuk-do			Gyeongsangnam-do			Jeju-do		
	SW14	SW49	SW50	SW53	SW57	SW58	SW60	SW64	SW72	SW75	SW80	SW98	SW104	SW105	SW106	SW107		
number of formulas	569	236	460	201	787	109	170	200	221	153	517	148	1246	1171	629	570		
m/z_{wa}	397.74	389.26	520.55	462.38	407.95	319.50	278.35	305.21	396.77	370.45	449.33	314.56	526.90	478.94	479.67	497.56		
C_{wa}	20.83	19.76	29.00	25.47	21.06	15.37	14.74	15.88	23.45	19.05	24.28	16.97	26.60	22.74	23.30	25.02		
H_{wa}	23.16	21.67	22.39	22.74	17.26	17.59	14.01	18.71	20.90	19.53	22.15	16.09	22.71	22.46	23.32	22.17		
N_{wa}	2.08	3.91	2.72	2.89	2.70	3.04	3.40	2.71	3.54	3.99	2.23	3.96	2.56	2.69	2.40	2.94		
O_{wa}	4.59	3.17	6.53	5.18	5.09	3.50	1.89	2.61	2.38	3.63	5.79	2.21	8.76	8.24	7.54	7.09		
S_{wa}	0.72	0.81	0.27	0.36	0.61	0.62	0.34	0.54	0.24	0.29	0.40	0.16	0.31	0.47	0.73	0.67		
H/C_{wa}	1.07	1.05	0.80	0.91	0.90	1.13	0.97	1.17	0.90	0.96	0.94	0.95	0.89	1.03	1.06	0.93		
O/C_{wa}	0.28	0.15	0.22	0.18	0.28	0.22	0.13	0.15	0.13	0.18	0.24	0.13	0.36	0.39	0.35	0.29		
N/C_{wa}	0.12	0.21	0.11	0.15	0.14	0.21	0.23	0.18	0.18	0.23	0.11	0.25	0.11	0.14	0.12	0.15		
S/C_{wa}	0.06	0.04	0.01	0.02	0.04	0.04	0.03	0.04	0.01	0.01	0.02	0.01	0.02	0.03	0.04	0.04		
DBE_{wa}	11.29	11.88	20.16	16.54	14.78	9.09	10.43	8.88	15.77	12.28	15.32	11.90	17.52	13.85	13.84	16.41		
$Almod_{wa}$	0.41	0.57	0.64	0.62	0.58	0.57	0.73	0.53	0.68	0.68	0.57	0.73	0.44	0.40	0.40	0.53		
CHO (%)	5.62	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00		
$CHON$ (%)	48.68	37.29	75.00	74.63	52.73	43.12	70.00	53.00	71.95	60.78	58.99	76.35	74.32	56.02	47.85	61.93		
$CHOS$ (%)	12.65	2.12	4.57	1.99	10.93	22.94	6.47	23.00	0.00	0.00	17.41	0.00	4.57	3.33	3.97	2.81		
$CHONS$ (%)	20.74	42.37	13.04	7.96	23.25	5.50	1.76	2.50	12.22	27.45	6.77	8.78	14.77	31.00	41.49	27.72		
Others (%)	12.30	18.22	7.17	15.42	13.09	28.44	21.76	21.50	15.84	11.76	7.35	14.86	6.34	9.56	6.68	7.54		
CAS (%)	17.75	11.02	39.78	12.94	35.20	7.34	20.00	13.00	15.38	22.22	26.89	18.92	25.68	21.18	19.55	26.67		
lignin/CRAM (%)	35.85	47.46	47.83	60.20	21.09	32.11	35.29	30.50	43.44	44.44	45.65	41.89	40.85	43.72	48.97	49.65		
protein (%)	2.28	1.69	0.00	0.00	0.25	0.92	0.00	11.50	0.00	0.00	12.96	0.68	0.40	7.09	9.86	4.04		
carbohydrate (%)	1.23	0.00	0.00	0.00	1.78	7.34	0.00	0.00	0.00	0.00	0.39	0.00	0.88	3.07	3.50	0.35		
lipid (%)	14.24	3.81	0.43	0.00	4.83	0.00	5.88	10.00	0.00	5.23	0.58	2.70	2.09	1.54	0.32	0.35		
UnsatHydroC (%)	14.76	36.02	11.96	26.87	21.09	47.71	38.82	32.50	41.18	26.80	13.54	35.81	11.88	8.63	10.81	13.33		
tannin (%)	6.33	0.00	0.00	0.00	13.72	4.59	0.00	0.00	0.00	1.31	0.00	0.00	17.26	14.18	7.00	5.61		
remainder (%)	7.56	0.00	0.00	0.00	2.03	0.00	2.50	0.00	0.00	0.00	0.00	0.00	0.96	0.60	0.00	0.00		

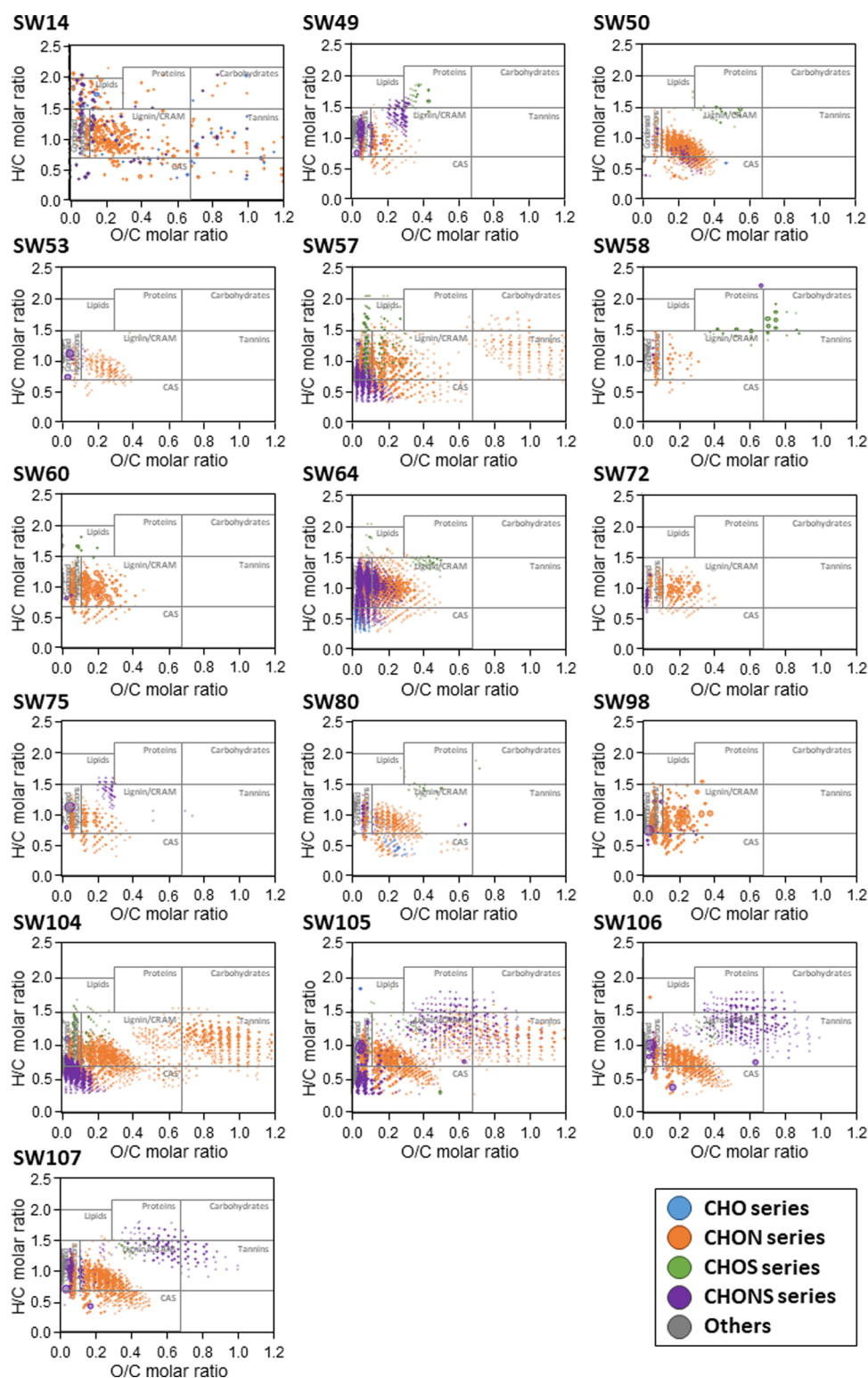


Figure 5. Van Krevelen diagrams of cold spring water samples from different sampling sites in South Korea.

CHONS. The high proportion of CHO was due to plants, algae, and sediment around the lake area. CHON composition showed the same results in this study compared to those of the bacterial activity of the sediment of the lake.³⁹ Spring water in Jeju-do had unique characteristics. The composition of the spring water samples was similar to that of other regions, but the concentrations were about 2 times higher than the average. Because Jeju-do is an island created by volcanic eruption of Halla

Mountain, the results likely reflect the characteristics of the basalt ground.⁴⁰ Also, basalt contains many pyroxenes and olivine sculptures. Pyroxene has a high content of calcium, magnesium, and iron.⁴¹

Lignin substances (41.81%) dominated the distribution of organic compounds in the van Krevelen diagrams, followed by UnsaturatedHydroC (24.48%), CASs (20.85%), and protein (3.23%). Lignin is found in the secondary cell wall of plants and is

Table 4. Molecular Characteristics of Hot Spring Water Samples from Different Sampling Sites in South Korea

sample	Gangwon-do TW02	Gyeonggi-do TW03	Incheon TW17	Chungcheongbuk-do TW04	Chungcheongnam-do TW07	Deajeon TW06	Gyeongsangbuk-do TW10	Gyeongsangnam-do TW14	Busan TW12
number of formulas	953	1457	273	877	1567	866	569	921	1088
m/z_{wa}	414.27	398.80	453.26	381.61	427.45	434.86	397.74	388.30	358.67
C_{wa}	21.23	18.22	25.22	18.11	19.29	23.01	20.83	19.65	18.46
H_{wa}	18.39	16.59	32.13	16.95	20.60	20.45	23.16	18.08	16.99
N_{wa}	1.95	2.57	1.87	2.43	2.62	2.19	2.08	1.95	2.44
O_{wa}	6.25	6.25	4.59	6.05	7.61	5.86	4.59	5.33	3.29
S_{wa}	0.46	0.89	0.62	0.55	0.56	0.46	0.72	0.71	1.07
H/C_{wa}	0.92	0.97	1.25	0.97	1.09	0.90	1.07	0.97	0.98
O/C_{wa}	0.34	0.37	0.25	0.34	0.43	0.30	0.28	0.32	0.20
N/C_{wa}	0.12	0.15	0.09	0.15	0.15	0.12	0.12	0.14	0.14
S/C_{wa}	0.03	0.06	0.04	0.04	0.03	0.03	0.06	0.05	0.07
DBE_{wa}	14.01	12.21	11.09	11.84	11.30	14.88	11.29	12.58	12.19
$Almod_{wa}$	0.49	0.46	0.31	0.49	0.33	0.53	0.41	0.46	0.53
CHO (%)	17.63	1.92	4.76	9.46	7.79	8.08	5.62	13.25	2.21
CHON (%)	43.97	32.53	44.69	47.43	39.89	49.88	48.68	25.62	21.14
CHOS (%)	4.20	7.55	7.69	6.50	5.74	3.70	12.65	14.98	12.78
CHONS (%)	25.71	48.66	24.18	26.00	43.20	29.56	20.74	35.29	43.29
others (%)	8.50	9.33	18.68	10.60	3.38	8.78	12.30	10.86	20.59
CAS (%)	36.10	30.40	17.22	26.68	18.12	39.84	17.75	29.75	23.81
lignin/CRAM (%)	33.79	34.11	21.98	39.00	47.80	37.30	35.85	36.16	30.42
protein (%)	4.83	1.99	5.49	1.14	10.40	3.58	2.28	4.99	0.00
carbohydrate (%)	1.89	3.29	2.56	1.48	3.57	0.92	1.23	3.37	1.38
lipid (%)	3.25	5.56	22.34	4.22	3.57	4.16	14.24	4.89	9.56
UnsattHydroC (%)	11.44	12.56	18.68	14.71	3.57	9.12	14.76	13.57	31.43
tannin (%)	4.51	7.07	2.56	8.44	10.66	1.50	6.33	3.15	0.64
remainder (%)	4.20	5.01	9.16	4.33	2.30	3.58	7.56	4.13	2.76

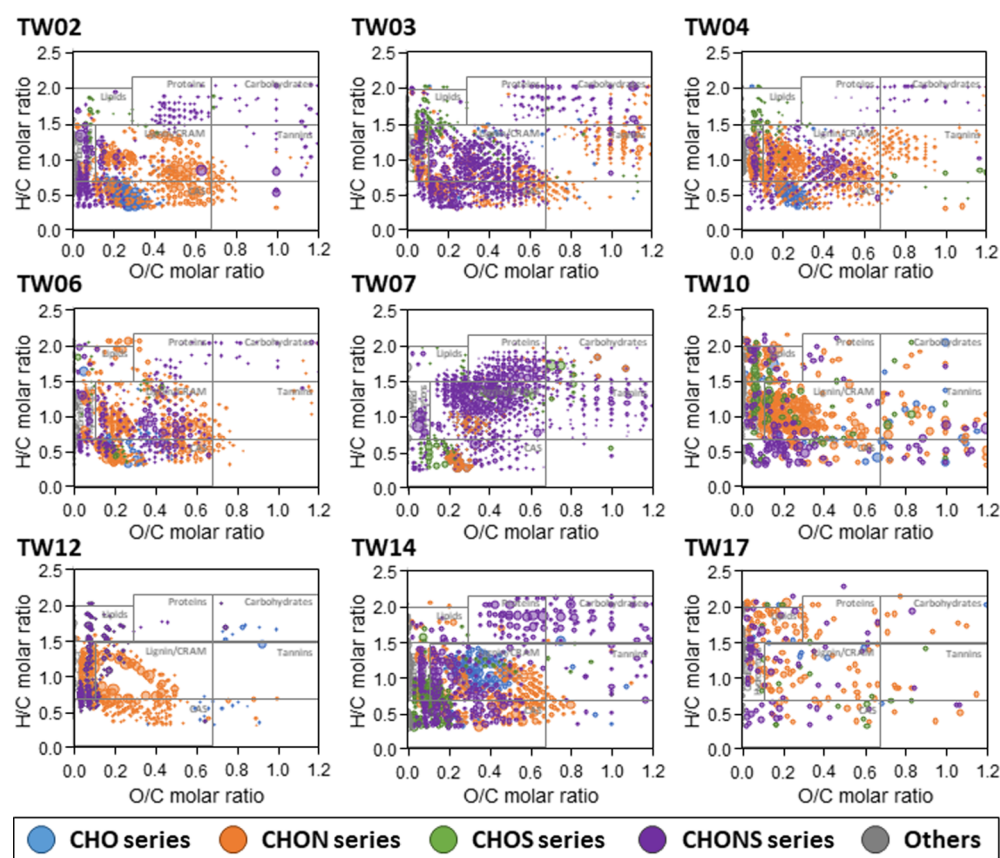


Figure 6. Van Krevelen diagrams of hot spring water samples from different sampling sites in South Korea.

recalcitrant to degradation.^{42,43} In addition, lignin lowers total cholesterol levels in lipidemic patients and has been studied to have anti-aging effects, which may be beneficial to health.^{44,45} CASs are also considered non-degradable.⁴⁶ The proportions of the lignin and CAS are likely the result of the locations of springs in South Korea, which are typically located in mountainous areas.⁴⁷ The van Krevelen diagrams and molecular properties of each sample are shown in Figure 5 and Table 3, respectively.

3.2.2. Hot Spring Water. We analyzed nine water samples from hot springs; the results are shown in Table 4. Overall, samples consisted mainly of CHON (39.31%), followed by CHONS (32.96%), others (11.45%), CHOS (8.42%), and CHO (7.86%). The composition was similar to that of cold spring water; however, hot spring water had about double the concentrations, including higher nitrogen and sulfur levels. The distribution of organic compounds in the van Krevelen diagram showed that lignin (35.16%) and CASs (26.63%) dominated, followed by UnsatHydroC (14.43%), lipids (7.98%), proteins (3.86%), and carbohydrates (2.19%); these trends differed from those of cold spring water samples. In hot springs, hot groundwater is supplied by acid gas and steam in deep aquifers, as confirmed by the detection of high sulfur and molecular ion contents. Differences in temperature, dissolved gas, and water properties in deep heat sources play important roles in water characteristics due to the presence of hydrogen sulfide,⁴⁸ which influences NOM.⁴⁹ For instance, Gonsior et al.²¹ analyzed four points at hot springs in Yellowstone National Park (WY, USA) and observed different trends depending on the site location, gas temperature, groundwater and surface water minerals, and heat source. Similarly, in this study, the hot spring water reflected the influence of both heat and spring water sources. In particular,

hot spring water was affected by the spring water source due to the characteristic mountainous terrain in South Korea. The van Krevelen diagrams and molecular properties of each sample are shown in Figure 6 and Table 4, respectively.

4. DISCUSSION

Basalt is a fine-grained igneous rock containing about 50% SiO₂. It also has a porous structure due to the process of its creation. Because of these characteristics, it becomes the basis for generating various organic matters. Microorganisms have been found to etch basalt glass within volcanic sediments, creating micron-sized tunnels and pits. Also, when the biological changes in the basalt glass dollop were analyzed by FT infrared (IR) spectroscopy, FTIR absorption bands consistent with aliphatic hydrocarbons, amides, esters, and carboxyl groups were confirmed. Also, it can represent the origin of the protein. These studies are evidence that can support the results showing high concentrations in basalt grounds.⁵⁰

The difference in the results of hot spring water and spring water indicates that the content of substances differs depending on the hot water. In order to discuss the results deeply, it is necessary to investigate the cause of the hot spring water. Hot springs occur mostly in volcanic terrain. There are hot springs in Japan in the active volcanic area, Yellowstone National Park in the USA, and geysers. The Republic of Korea does not have active volcanoes, and its cause is different from that of hot spring water in volcanic areas.

Pollack et al. (1993) report that the source of geothermal heat is about 40% due to the decay heat of radioactive elements present in rocks.⁵¹ The direct heat source appears mainly in the volcanic zone and the boundary surface between the plates like

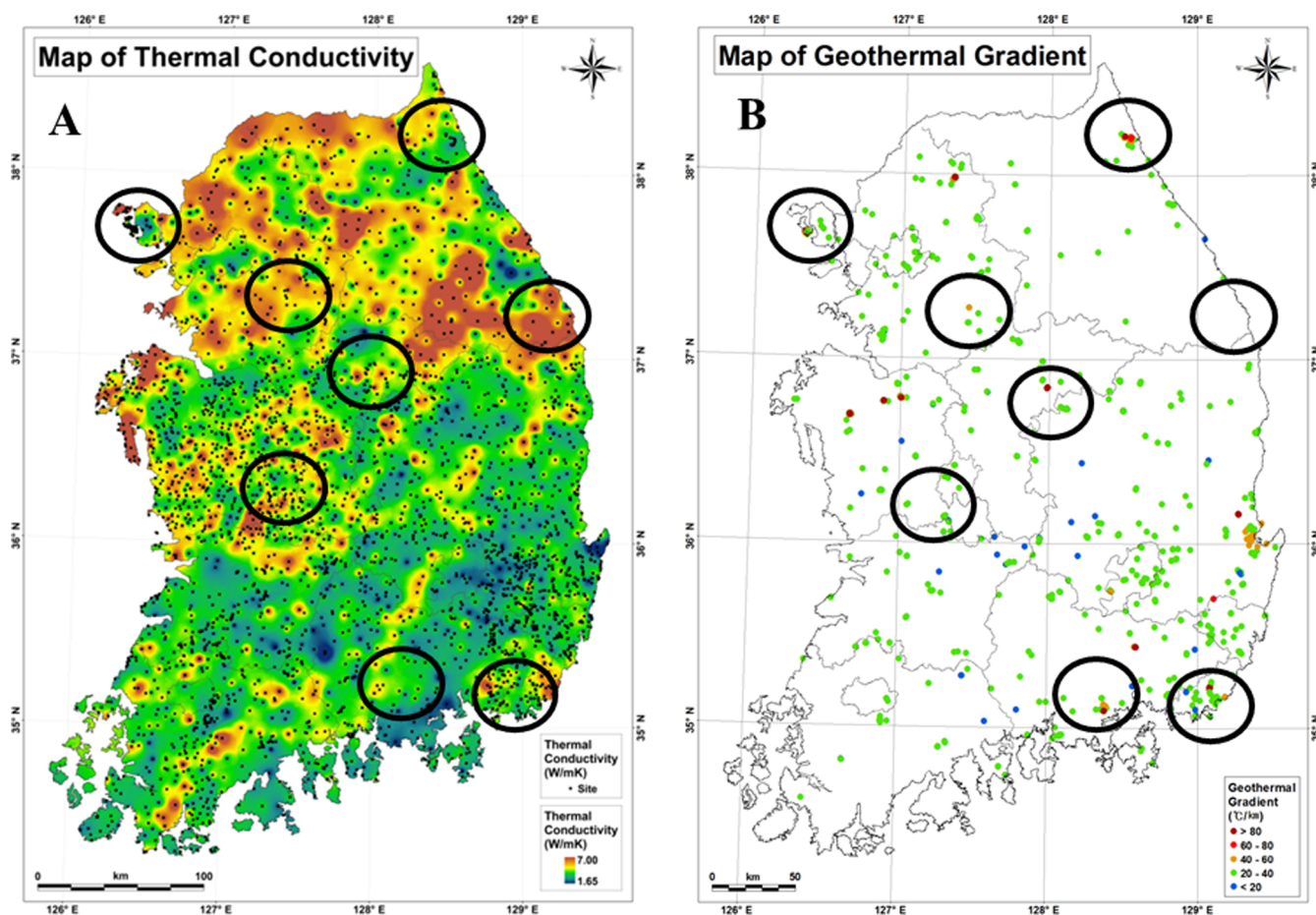


Figure 7. Distribution of rock thermal conductivity and borehole-temperature detection location and the geothermal gradient distribution map in Korea [(A) distribution of rock thermal conductivity and (B) borehole-temperature detection location and the geothermal gradient distribution map. Black circle: hot spring area].

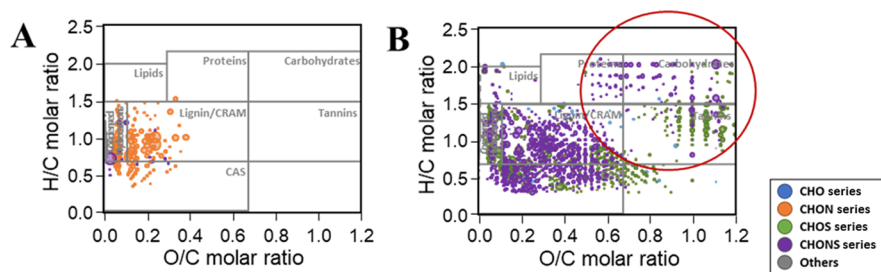


Figure 8. Comparison of van Krevelen diagrams of spring water and hot spring water [(A) spring water and (B) hot spring water].

the Pacific Rim Tectonic Zone in the world, and it is a very small area in the whole area.

Full-scale geothermal research in Korea began with joint research between Korea and Japan,⁵² and in order to measure the amount of geothermal heat flow, a borehole must be provided, and temperature measurement and thermal conduction of rocks must be measured within the borehole. Looking at Figure 7, we compare the location of hot springs based on the distribution of rock thermal conductivity, borehole-temperature detection location, and geothermal gradient distribution in Korea, and we can confirm that they are quite similar.

Upon comparing the van Krevelen diagrams of spring water and hot spring water, we can see that in the case of hot spring water, more NOM and a wider range of detection are found. Also, you can see a specific pattern. This is judged to be NOM

produced by the generation of hot temperature as a characteristic of hot spring water and the oxidation of minerals. A specific pattern is shown in Figure 8. Based on these results, we scientifically confirmed through a high-resolution mass spectrometer the fact that spring water and hot spring water are clearly different types of water in the pattern of NOM detected.

5. CONCLUSIONS

We analyzed cold and hot spring water samples collected from various regions of South Korea and characterized the molecular composition of NOM in water using a luminescence spectrometer and 15T FT-ICR-MS. The proportions of lignin and CASs were identical in cold and hot spring waters due to the geographic characteristics of South Korea. Notably, high NOM concentrations were detected in Jeju-do due to the volcanic

characteristics of the region. These samples also contained a high concentration of organic substances due to the nature of the hydrothermal water, consistent with the van Krevelen diagram results. This study establishes an analytical method to evaluate the characteristics of water, which can be used as the basis for future research on water-related environmental problems and diseases.

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Notes

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