温泉科学（J. Hot Spring Sci.），75，62-79（2025）

原　　著

Geochemical Characteristics of Kuroyu Hot Springs in the Yokohama-Kawasaki Area, and the Sources of Organic Components

Genki I. Matsumoto[1) 2)]* and Miho Komesu[1)]

(Received August 31, 2025, Accepted September 12, 2025)

横浜・川崎地域における黒湯の地球化学的特徴と有機成分の起源

井上源喜[1) 2)]*，米須美穂[1)]

要　　旨

わが国には腐植物質を含む黒湯温泉やモール温泉と呼ばれる透明で茶褐色や黒褐色の温泉が多数分布するが，その地球化学的特徴はほとんど研究がなされていない．本研究では神奈川県横浜・川崎地域における黒湯温泉の地球化学的研究を行い，無機化学成分，脂質バイオマーカーおよびフミン酸の熱分解生成物中の脂肪酸の特徴などを明らかにし，それらの起源生物および堆積環境の解明を行った．泉質は主としてナトリウム—炭酸水素塩冷鉱泉またはナトリウム—炭酸水素塩・塩化物冷鉱泉であった． Log 透視度とフミン酸濃度の逆相関関係より，黒湯の着色レベルはフミン酸含有量によることが判明した．

酢酸エチル抽出物中の飽和脂肪酸は，主成分が短鎖 *n*-アルカノイック酸（C_{12}-C_{18}）で，少量の長鎖 *n*-アルカノイック酸（C_{20}-C_{34}）および分岐脂肪酸（イソ，アンチイソ-C_{13}-C_{17}）であった．想定外であるがかなりの量の直鎖一不飽和脂肪酸（*n*-$C_{16:1}$(9)，炭素数：不飽和数（二重結合位置），*n*-$C_{16:1}$(8?)，*n*-$C_{16:1}$(7)，*n*-$C_{16:1}$(5)，*n*-$C_{18:1}$(9)，*n*-$C_{18:1}$(7)）が含まれていた．ステロールはコレステロールが主成分であった．フミン酸の熱分解 tetramethylammonium hydroxide メチル化生成物中の脂肪酸の特徴は，酢酸エチル抽出物の特徴と同様であった．これらの有機成分の主要な起源は，水深 25～50 m の上総層群鶴川層または半深海の陸棚斜面（三浦層群池子層）におけるプランクトンを含む藻類で，少量の維管束植物および真正細菌によると判断される．多不飽和脂肪酸は堆積環境で分解除去され，一不飽和酸脂肪酸のみが残存していると考えられる．堆積年代は上総層群が初期更新世の 2.58～0.77 Ma，三浦層群が後期鮮新世の 3.60～2.58 Ma と考えられる．

[1)] Department of Environmental Science, Faculty of Social Information Studies, Otsuma Women's University, 2-7-1, Karakida, Tama-shi, Tokyo 206-8540, Japan. [1)] 大妻女子大学社会情報学部環境情報学専攻　〒206-8540　東京都多摩市唐木田 2-7-1.

[2)] Current address: Institute of Human Culture Studies, Otsuma Women's University, 12, Sanban-cho, Chiyoda-ku, Tokyo 102-8357, Japan. *Corresponding author：E-mail genki@otsuma.ac.jp, TEL +81-3-5275-6047, FAX +81-3-3222-1928. [2)] 現住所：大妻女子大学人間生活文化研究所　〒102-8357　東京都千代田区三番町 12.

キーワード：横浜・川崎地域，黒湯，バイオマーカー，フミン酸，熱分解 TMAH メチル化，堆積環境

Abstract

Geochemical studies on Kuroyu hot springs in Yokohama-Kawasaki area of Kanagawa Prefecture were carried out to elucidate their characteristics, especially focused on organic components including humic acid, together with source organisms and sedimentary environments. Kuroyu hot spring qualities were mainly sodium-hydrogen carbonate or sodium-hydrogen carbonate/chloride. The inverse correlation between Log-transparency and humic acid contents showed that color levels of Kuroyu hot springs are attributed to humic acid contents. Saturated fatty acids were mainly composed of short-chain *n*-alkanoic acid (C_{12}-C_{18}) with small amounts of long-chain *n*-alkanoic acids (C_{20}-C_{34}) and branched acids (*iso*- and *anteiso*-C_{13}-C_{17}). Unusually, considerable amounts of *n*-alkenoic acids (n-$C_{16:1}$(9), carbon chain length : number of unsaturation (double bond position), *n*-$C_{16:1}$(8?), *n*-$C_{16:1}$(7), *n*-$C_{16:1}$(5), *n*-$C_{18:1}$(9), *n*-$C_{18:1}$(7)) were found in ethyl acetate extracts and pyrolysis-tetramethylammonium hydroxide-methylation products (Py-TMAH-Me) of humic acid. Major sterols in ethyl acetate extracts were all cholesterol with small amounts of 24-methylcholesterol and 24-ethylcholesterol and others. Organic components in Kuroyu hot springs are mainly originated from algae including plankton with small contribution of vascular plants and bacteria. The absence of polyunsaturated fatty acids is ascribed to the degradation loss in the sedimentary environments. Well depths (70–200 m) except for YK08 sample (1,500 m) showed that sedimentary environments are the Tsurukawa formation of Kazusa group formed in the Early Pleistcene (2.58–0.77 Ma) with marine water depth of 25–50 m, and the Ikego Formation of Miura Group (YK08 sample) formed in the Late Pliocene (3.60–2.58 Ma) of upper and middle bathyal zone environment.

Key words : Yokohama-Kawasaki area, Kuroyu hot spring, biomarker, humic acid, pyrolysis-TMAH-methylation, sedimentary environments

1. Introduction

Many hot springs containing humic substances such as humic and fulvic acids, so-called Kuroyu hot springs (black springs) and/or Moal hot springs are widely distributed throughout Japan. Especially, they are found in certain plain areas of Hokkaido, Aomori, Akita and Yamagata Prefectures of Tohoku region, Saitama, Chiba and Kanagawa Prefectures and Tokyo of the south Kanto region, Niigata and Gifu Prefectures of Chubu region, Osaka Prefecture of Kinki region, Miyazaki and Kagoshima Prefectures of Kyushu region (Kanroji, 2010; Matsumoto *et al.*, 2020). These colored hot springs containing humic substances are associated with the area of water-soluble natural gas deposit (Petroleum Technology Association, 1983; Kanroji, 2010) as well as peat land in Japan. Humic substances, formed by diagenesis of biological materials, are chemically unidentified complex mixture of polymers (Ishiwatari, 2008; Kanroji, 2010).

Very little is known on organic components in Kuroyu and Mohl hot springs, and thus these studies are strongly required in hot spring science and geochemistry. We studied fatty acids and sterols in ethyl acetate extracts (Matsumoto *et al.*, 2020) and fatty acids in humic acid by pyrolysis-tetramethylammonium hydroxide methylation gas chromatography/mass spectrometry (Py-TMAH-Me-GC/MS) in Kuroyu hot springs from Ohta-ku in Tokyo (Matsumoto and Ohmiya, 2019; Matsumoto *et al.*, 2020). Pyrolysis cut chemical bonds of ethers, esters and amides, and/or cause decarboxylation and dehydration reactions. TMAH forms methyl esters and/or methyl ethers of pyrolyzed product, and GC/MS determines esters and/or ethers with molecule levels. Py-TMAH-Me-GC/MS needs very small amounts of samples without any pretreatment, and very useful for the study of organic components in humic substances (Yamamoto *et al*., 2007; Matsumoto and Ohmiya, 2019).

Here we studied geochemical characteristics, especially biomarkers such as fatty acids and sterols in ethyl acetate extracts and in humic acids by Py-TMAH-Me-GC/MS in Kuroyu hot springs (Sentous) from the Yokohama-Kawasaki area in Kanagawa Prefecture to elucidate their geochemical features and sources as well as sedimentary environments.

2. Materials and Methods

2.1 Kuroyu hot spring samples

Tea brown and black brown with transparent groundwaters are widely distributed in Yokohama-Kawasaki area in Kanagawa Prefecture (Fig. 1). In August 22, 24, 2017 and September 18, 2017, Kuroyu hot springs (YK01-YK10) were sampled from hot water supply port of Kuroyu hot springs in 2 L polyethylene bottles from the Yokohama-Kawasaki area (Fig. 1, Table 1). The Kuroyu hot springs were kept in a refrigerator until analyses. YK01-YK10 samples are same as Sta.1–Sta.10 samples, respectively, reported by Takano *et al*. (2020)

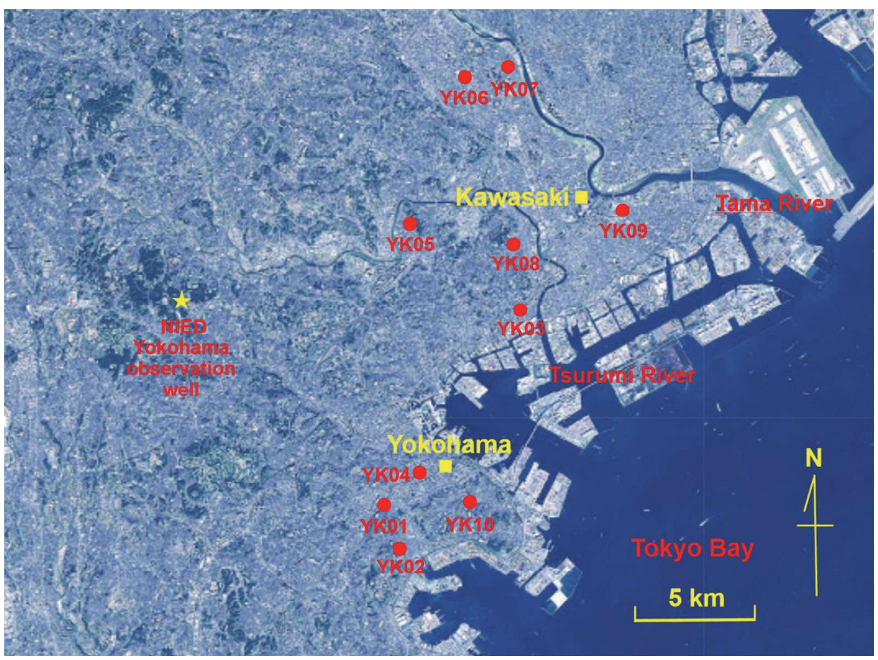

Fig. 1 Sampling sites of Kuroyu hot springs (Sentou, YK01-YK10) in the Yokohama-Kawasaki area (Geographical Information Authority of Japan. https://maps.gsi.go.jp/development/ichiran.html (Data source: GSI, TSIC, GEO Grid/AIST, courtesy of the U.S. Geological Survey, GEBCO))

Table 1　Physico-chemical properties of Kuroyu hot springs from the Yokohama-Kawasaki area in Kanagawa Prefecture, Japan

Sample No.	Sampling date	Water temp. (℃) *	pH*	Electric conductivity (mS/m)*	Total dissolved matter (g/kg)*	Well depth (m)*	Trans-pererency (cm)	Humic acid (mg/L)	Hot spring quality
YK01	Aug. 24, 2017	18.9	8.40	146	1.524	130	13.4	118	Na-HCO_3
YK02	Aug. 22, 2017	17.0	8.29	121	1.345	200	13.7	126	Na-HCO_3
YK03	Aug. 22, 2017	16.5	8.40	113	1.074	152	6.2	164	Na-HCO_3
YK04	Aug. 22, 2017	18.4	7.50	190	1.596	70	23.0	102	Na-HCO_3・Cl
YK05	Aug. 24, 2017	17.4	7.93	134	1.508	80	8.6	199	Na-HCO_3
YK06	Aug. 24, 2017	19.4	8.20	88	1.074	170	>30	30	Na-HCO_3
YK07	Aug. 22, 2017	19.2	8.30	186	2.057	160	15.7	122	Na-HCO_3
YK08	Sept. 18, 2017	22.3	7.80	-	17.63	1,500	>30	76	Na-Cl
YK09	Aug. 24, 2017	17.4	7.48	820	5.978	85	>30	57	Na-Cl
YK10	Aug. 24, 2017	16.8	8.68	105	1.035	70	29.3	86	Na-HCO_3
Average±SD#		18.0±1.1	8.10±0.51	211±230	1.910±1.560	124±49		112±51	

*Hot spring data sheet and personal communication.

#Standard deviation. YK08 sample was excluded for the calculation of average and SD.

2. 2　Analytical methods

1)　Transparency and humic acid

Transparency was measured with a 30 cm transparency meter (Shibata Science Co. Ltd.). Humic acid contents were determined by the method of Guidelines for Mineral Spring Analysis (Natural Environment Bureau, Ministry of the Environment, 2014). Kuroyu hot springs (each 300 mL) were treated in a 300 mL glass beaker, and added 5 mL concentrated hydrochloric acid to precipitate humic acid, and then to stand for overnight at room temperatures. Humic acid was precipitated completely and transparent water were obtained (Fig. 2A, 2B). Humic acid was collected by filtration through glass fiber paper (Whatman, GF/C pore size 1.2 μm, pretreated 500℃ for 2h in an electric furnace, weight measured) attached in all glass filtering apparatus under reduced pressure. No pure water wash was done to avoid redissolution of humic acid. Water and hydrochloric acid on glass fiber paper was evaporated under reduced pressure with an aspirator in a screw-in desiccator at 40℃ in water bath. After confirmation of no hydrochloric acid odor of glass fiber filters, these filters were kept in a desiccator with drying agent of silica gel for overnight, and weighed humic acid contents. The filters packed in aluminum foil were kept in a freezer at −28℃ until Py-TMAH-Me-GC/MS.

2)　Lipid biomarker in ethyl acetate extract

Analytical methods of lipid biomarkers (hydrocarbon, fatty acids and sterols) are shown elsewhere (Matsumoto *et al.* 1979, 1982, 2003; Matsumoto and Watanuki, 1992). Briefly, Kuroyu hot springs (each 300 mL) were acidified with hydrochloric acid (pH<2), and extracted 3 times with ethyl acetate (50 mLx3). The ethyl acetate extracts were concentrated to dryness and saponified with 0.5 mol/L potassium hydroxide/methanol (2 mL, 80℃, 2 h). The ethyl acetate extracts were fractionated by column chromatography on a silica gel column (160 mm×6 mm i.d., 100 mesh, 5% water). Hydrocarbon and fatty acid-sterol fractions were obtained by the elution with hexane and ethyl acetate, respectively. A half volume subsample of the fatty acid-sterol fraction was methylated with diazomethane. The other half volume of the fatty acid-sterol

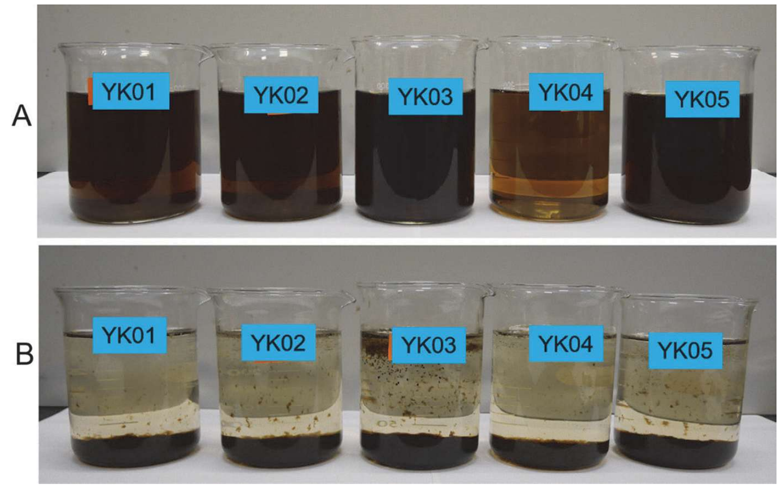

Fig. 2A　Kuroyu hot springs (YK01-YK05) from the Yokohama-Kawasaki area.
A: Before addition of hydrochloric acid.　B: Precipitation of humic acid after addition of hydrochloric acid.

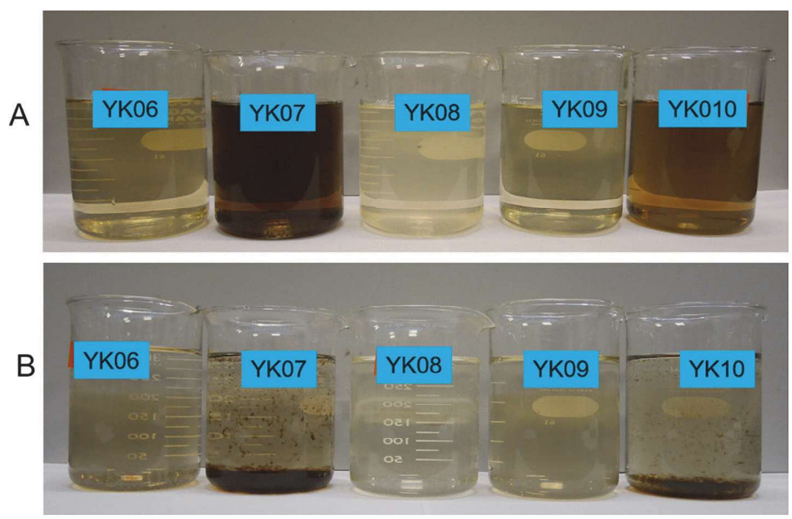

Fig. 2B　Kuroyu hot springs (YK06-YK10) from the Yokohama-Kawasaki area.
A: Before addition of hydrochloric acid.　B: Precipitation of humic acid after addition of hydrochloric acid.

fraction was trimethylsilylated (TMS) with 25% *N,O*-bis(trimethylsilyl acetamide) acetonitrile solution, to obtain sterol TMS derivatives.

Hydrocarbons, fatty acid methyl esters and sterol-TMS derivatives were analyzed by a JEOL Q1000 GC/MS equipped with a fused silica capillary column (J&W DB5 ms, 30 m, 0.25 mm i.d., film thickness 0.1 μm). Splitless mode was employed. Column oven temperature was programmed from 70 to 120℃ at 30℃/min, from 120 to 320℃ at 8℃/min and kept at 320℃ for 3.5 min. The flow rate of helium carrier gas was 1.2 mL/min. The temperatures of injector, interface and ion source were maintained at 300, 300 and 250℃, respectively. Ionization energy, filament current and detector voltage were 70 eV, 200 μA and -1,000 V, respectively.

3) Py-TMAH-Me-GC/MS of humic acid

Glass fiber filter retained humic acid was cut (ca.10 × 2 mm) with scissors. Py-TMAH-Me-GC/MS was carried out by the method reported elsewhere (Matsumoto and Ohmiya, 2019). Cut glass fiber paper was wrapped in pyrofoil (curie point at 445℃), together with TMAH reagent (25% tetramethylammoniumhydroxide methanol 10 μL) and nonadecanoic acid (10 ng) as internal standard. The solvent was removed by retained heat of the GC/MS column oven. Py-TMAH-Me-GC/MS was carried out by a JAI-5 Curie point pyrolyzer at 445℃ connected directly with a JEOL Q1000 GC/MS. Oven and needle temperatures of the pyrolyzer were kept at 398 and 250℃, respectively. Column oven temperatures were programmed from 60 to 320℃ at 8℃/min and kept at 320℃ for 2.5 min. Split mode with split ratio of 1/50 was employed. Other GC/MS conditions were same as the lipid biomarker analysis stated above.

3. Results and Discussion

3.1 Sedimentary environment in the Yokohama-Kawasaki area

Ozawa and Eto (2005) investigated geology and underground geological structure for drilling samples and data from 26 deep wells; 24 wells with the depth more than 1000 m and two wells less than 1000 m in the central and eastern parts of Kanagawa Prefecture. In the Yokohama and Kawasaki region, they suggested that hot springs are distributed in the Kazusa and Miura Groups. Well depths of YK01-YK07 and YK09-YK10 range from 70–200 m with an average of 124 ± 49 m, while YK08 is much deep of 1,500 m (Table 1). Takano *et al.* (2020) discussed 9 samples of YK01-YK07 and YK09-YK10 are spring out from Tsurukawa Formation of Kazusa Group, while YK08 sample is spring out from Ikego Formation of Miura Group. Kazusa Group forms in the Early Pleistocene (2.58–0.77 Ma). Tsurukawa Formation forms in water depth of 25–50 m of marine environment estimated by molluscan fossils groups (Baba, 2015). Ikego Formation of Miura Group (YK08) forms in Late Pliocene (3.60–2.58 Ma) marine deposit of Upper to Middle bathylal zone environment (Eto, 1986). Here we added age spans in this area based on International Commission Stratigraphy (2024). Kuroyu sampling sites of Yokohma-Kawasaki area are located at a part of Minami Kanto gas field (Geological Survey of Japan, 1976; Daita *et al.*, 2007; Daita and Odawara, 2014).

Although Ohta-ku area in Tokyo is located in a Minmi-Kanto gas field, sedimentary environments are considerable different because well depths of Ohta-ku area are ranging from 30 to 120 m with an average of 86 ± 30 m (Matsumoto *et al.*, 2020) which is much shallower than those of Yokohama-Kawasaki area (124 ± 49 m, Table 1), and thus, sediment layers consist of Shimousa and Kazusa Groups with ages from 0.50 to 1 Ma (Matsumoto *et al.*, 2020). Shimousa Group is formed in Paleo Tokyo Bay of shallow marine environments (Kikuchi, 1980)., while Kazusa Group is formed in the lower, middle and upper marine land shelf slopes (Sekimoto *et al.*, 2009).

3.2 Hot spring quality

Table 1 shows physico-chemical properties of Kuroyu hot springs from the Yokohama-

Kawasaki area in Kanagawa Prefecture. Water temperature, pH, electric conductivity, total dissolved matter and well depth were used with the hot spring analysis data sheet of each Kuroyu hot spring and personal communication of the staff. Also, Takano *et al.* (2020) described water temperature, pH and well depths. Water temperatures ranging from 16.5 to 18.7℃ with an average of 17.5±0.6 (standard deviation) showed all cold mineral springs. Here, we excluded YK08 sample because well depth (1,500 m) and total dissolved matter (17.63 g/kg) were much greater than the others (Table 1). pH ranging from 7.50 to 8.68 with an average of 8.10±0.51 showed all weak alkaline reflecting major hot spring quality of sodium-hydrogen carbonate, sodium-hydrogen carbonate・chloride or sodium chloride (Table 1). These water qualities of Yokohama-Kawasaki area are generally similar to those of Ohtaku area in Tokyo (Matsumoto and Ohmiya, 2019; Matsumoto *et al.*, 2020), since both areas are located in the Minami-Kanto gas field (Geological Survey of Japan, 1976; Daita and Odawara, 2014). Well depths ranged from 70 to 200 m in Yokohama-Kawasaki area (Table 1) correspond to the Tsurukawa Formation of Kazusa Group formed in water depth of 25–50 m of marine environment (Baba, 2015).

Most predominant cation was Na^+ greater than 80% in all the Kuroyu hot springs. Most predominant anion of HCO_3^- greater than 80% was found in YK01-YK03, YK05-YK07 and YK10 samples, while YK04 sample contain major anion of HCO_3^- 61.15% and of Cl^- 38.65%. Cl^- is the most predominant anion in YK08 and YK09 samples (Table 1). They are somewhat different from the Ohta-ku area in Tokyo (Matsumoto *et al.*, 2020), although major cation (Na^*) and major anion (HCO_3^- or Cl^-) is similar to etch other.

It is well known that alkaline sodium-hydrogen carbonate hot springs with low concentration and low temperatures are widely distributed in granite and/or rhyolite zones, *etc.* (Mashiko *et al.*, 1959: Suzuki, 1979; Takamatsu *et al.*, 1981; Seki *et al.*, 2004; Muramatsu *et al.*, 2008). Granite consists of major components of island arc and land, and is widely distributed in Japan. Matsumoto *et al.* (2020) discussed mechanism of the formation of alkaline pH, dominance of Na^+ cation and the dominance of HCO_3^- and/or Cl^- anions in Kuroyu hot springs in the Ohta-ku area, and thus similar processes occurred in the Yokohama-Kawasaki area.

3.3 Transparency and humic acid contents

Very little is known on transparency of Kuroyu hot springs. Transparency of Kuroyu hot springs from Yokohama-Kawasaki area varied widely from 6.2 cm to ＞30 cm (Table 1). This result is somewhat higher than those of Ohtaku area 3.0–＞30 cm (Matsumoto and Omiya, 2009; Matsumoto *et al.*, 2020). Humic acid contents showed wide range of variation ranging from 30 to 199 mg/L with an average of 112±51 mg/L (Table 1) which is somewhat lower than those of Ohta-ku area samples ranging from 5.8 to 325 mg/L with an average of 126.58±104.29 mg/L (Matsumoto and Ohmiya, 2019; Matsumoto *et al.*, 2020). Kanroji (1999a, 1999b, 2010) reported humic acid contents in Tokyo including Ohta-ku area since 1950th and obtained less than 1 mg/L–490 mg/L. Humic acid contents of Kuroyu hot springs in Tokyo Bay area including Chiba Prefecture are 0.34–194 mg/L (Imahashi, 2015). They are probably local variation in the Minami Kanto gas field (Geological Survey of Japan, 1976; Daita *et al.*, 2007; Daita and Odawara, 2014). In reference, humic acid contents of Mohl hot springs in Hokkaido are 0.2–70 mg/kg (Aoyanagi *et al.*, 2005; Takano *et al.*, 2016).

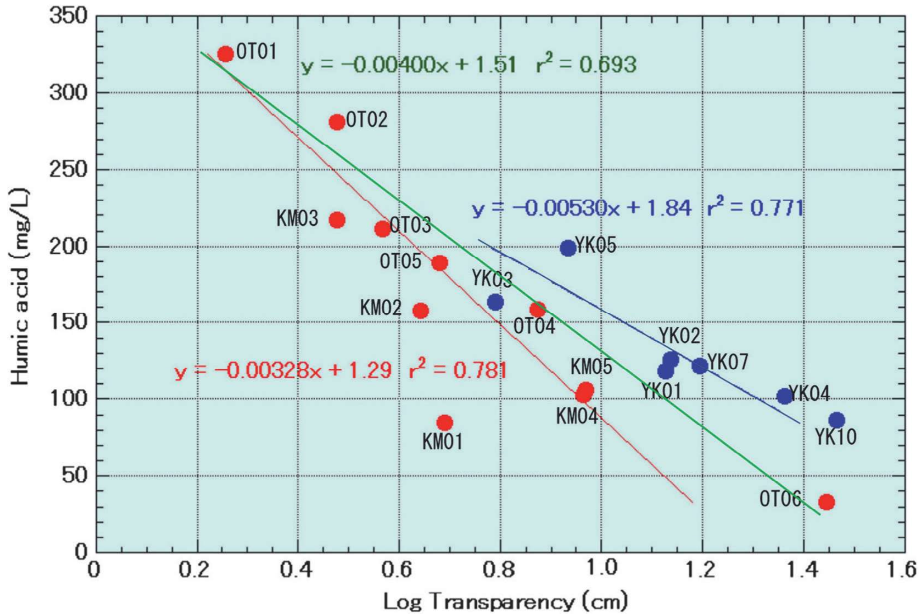

Fig. 3 Correlation between Log-transparency and humic acid contents in Kuroyu hot spring samples from the Yokohama-Kawasaki area (YK01-YK10, blue circle), as compared with those of the Ohta-ku area (OT01-OT06, red circle) including Kamata samples (KM01-KM05, red circle, Matsumoto and Ohmiya, 2019; Matsumoto *et al*., 2020). Correlation coefficients of Yokohama-Kawasaki area (blue line) and Ohta-ku area (red line) were r^2=0.771 and 0.781, respectively. Combined whole samples (green line) demonstrated a correlation coefficient of r^2= 0.693.

Figure 3 shows relationship of Log-transparency and humic acid contents of the Yokohama-Kawasaki area, as compared with those of Ohta-ku area, and combined whole samples of Yokohama-Kawasaki area, and Ohta-ku area. The Log-transparency and humic acid contents from the Yokohama-Kawasaki areas (YK01-YK10, blues circle) were inversely correlated with relatively high correlation coefficient of r^2=0.771 (Fig. 3). Those of Ohta-ku area (including Kamata samples, OT01-OT06, KM01-KM06, red circle) were similar to the Yokohama-Kawasaki area with a correlation coefficient of r^2=0.781. Combined whole samples (green line) demonstrated a correlation coefficient of r^2=0.693). These results revealed that humic acid contents reflect color level of Kuroyu hot springs in the Minami-Kanto gas field.

3.4 Features of fatty acids and sterols

3.4.1 Fatty acids and sterols in ethyl acetate extracts

Hydrocarbons were very low concentrations and not detected in all the Kuroyu hot springs. Normal-alkanoic acids ranging in carbon-chain length from *n*-C_{12} to *n*-C_{32} with a predominance of even-carbon numbers were found in YK01-YK10 samples (except for YK08), together with branched (*iso*- and *anteiso*-$C_{13\text{-}17}$), and mono-unsaturated fatty acids ($C_{16:1}$ and $C_{18:1}$, carbon chain length: number of unsaturation, Table 2). To determine double bond position of alkenoic acids, we calculated equivalent carbon chain length (ECL) of the methyl esters of fatty acids according to Christie (1989) and Matsumoto *et al*. (2020), as identified to *n*-$C_{16:1}$ (9, double bond position), *n*-$C_{16:1}$

Table 2 Fatty acid composition of ethyl acetate extracts in Kuroyu hot springs from the Yokohama-Kawasaki area

Sample No.	YK01	YK02	YK03	YK04	YK05	YK06	YK07	YK08	YK09	YK10	Average±SD*
n-Short (%)											
12	2.95	10.12	9.68	7.61	3.43	6.95	5.27	5.75	3.65	11.43	
13	0.34	0.55	0.15	0.35	0.28	0.61	0.46	0.61	0.24	0.30	
14	12.62	10.69	8.77	9.66	10.79	10.01	11.20	10.14	11.86	9.15	
15	1.62	2.84	3.63	2.02	1.77	4.00	5.27	4.73	3.95	2.33	
16	16.79	35.15	37.79	36.59	20.43	34.76	41.18	42.25	38.02	28.58	
17	0.53	0.79	1.15	0.64	0.59	1.31	1.38	1.62	1.22	0.73	
18	3.09	6.75	6.05	6.44	5.56	7.23	8.57	7.44	13.08	5.03	
19	0.13	0.12	0.26	0.09	0.10	0.29	0.31	0.36	0.19	0.15	
Toal *n*-short (%)	38.07	67.00	67.47	63.41	42.95	65.17	73.65	72.89	72.22	57.70	60.85±12.50
n-Long (%)											
20	0.35	0.54	0.65	0.59	0.43	0.73	0.71	0.88	0.51	0.49	
21	0.10	0.14	0.23	0.11	0.08	0.14	0.12	0.21	0.08	0.12	
22	0.40	0.54	0.66	0.40	0.53	0.61	0.54	0.69	0.41	0.50	
23	0.16	0.16	0.35	0.17	0.19	0.32	0.43	0.35	0.18	0.20	
24	0.67	0.90	1.16	0.70	0.94	0.53	1.37	1.62	0.39	1.01	
25	0.14	0.20	0.32	0.16	0.17	0.30	0.35	0.42	0.22	0.20	
26	0.46	0.67	0.85	0.37	0.85	0.61	0.53	0.81	0.30	0.55	
27	0.09	0.12	0.26	0.06	0.18	0.12	0.29	0.15	0.07	0.10	
28	0.39	0.68	0.88	0.28	1.15	0.40	0.16	0.33	0.15	0.33	
29	0.11	0.15	0.16	0.08	0.27	0.15	0.18	0.18	0.00	0.12	
30	0.25	0.52	0.65	0.27	1.15	0.26	0.40	0.21	0.00	0.28	
31	0.04	0.09	0.10	0.04	0.21	0.09	0.43	0.00	0.00	0.03	
32	0.10	0.30	0.33	0.11	0.71	0.20	0.47	0.00	0.00	0.13	
Total *n*-long (%)	3.11	4.62	6.17	3.20	5.95	4.17	5.08	5.85	2.30	3.90	4.28±1.31
Branched (%)[$]											
i12	0.62	0.31	0.48	0.29	0.16	1.33	0.10	0.95	0.24	0.09	
i13	0.13	0.17	0.12	0.06	0.05	0.22	0.49	0.30	0.06	0.17	
i14	0.70	0.96	0.97	0.64	0.41	1.06	0.72	1.15	0.43	0.55	
i15	2.69	1.21	0.42	0.29	0.26	0.70	0.26	0.41	0.18	0.27	
i16	0.87	0.62	0.73	0.41	0.39	1.17	1.22	1.28	0.79	0.55	
i17	0.20	0.11	0.15	0.09	0.10	0.11	0.10	0.17	0.09	0.06	
a13	0.09	0.22	0.15	0.12	0.08	0.14	0.40	0.17	0.06	0.09	
a15	1.07	1.10	0.73	0.47	0.49	1.00	0.86	0.95	0.46	0.50	
a17	0.16	0.20	0.24	0.12	0.15	0.31	0.33	0.41	0.24	0.18	
Total branched (%)	5.92	4.58	3.51	2.20	1.93	4.70	4.38	4.83	2.31	2.38	3.55±1.42
n-Alkenoic (%)[#]											
16:1(9)	17.11	7.48	4.44	11.18	16.90	9.06	6.13	5.95	3.04	6.72	
16:1(8?)	20.95	7.03	5.33	12.47	14.42	7.67	0.42	0.68	10.71	8.05	
16:1(7)	3.84	1.69	2.67	2.17	5.03	1.53	0.20	0.81	0.91	7.77	
16:1(5)	5.76	2.81	0.45	3.45	7.45	0.92	0.00	1.01	0.49	0.67	
18:1(9)	1.75	3.71	9.07	0.86	4.41	5.62	9.22	6.96	6.69	10.11	
18:1(7)	3.49	1.07	0.89	1.05	0.96	1.15	0.92	1.01	1.34	2.70	
Total *n*-alkenoic (%)	52.89	23.79	22.85	31.19	49.18	25.96	16.89	16.43	23.18	36.02	31.33±12.44
Total (%)	100	100	100	100	100	100	100	100	100	100	

*Standard deviation. YK08 sample was excluded for the calculation of average and SD.

[$]i and a are *iso*- and *anteiso*-branched acids, respectively.

[#]Carbon chain length: number of unsaturation (double bond position).

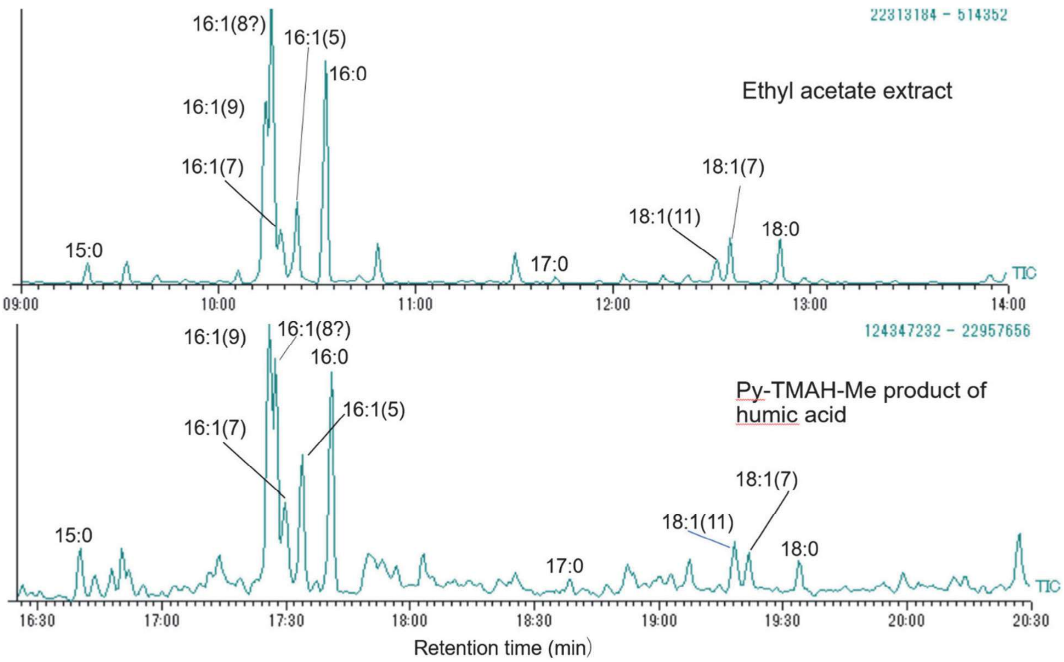

Fig. 4 Partial gas chromatograms of double bond position of methyl esters of *n*-alkenoic acids in ethyl acetate extract and Py-TMAH-Me products of humic acid from Kuroyu hot springs in the Yokohama-Kawasaki area, Japan. m: n (x)＝Carbon chain length: double bond (double bond position).

(8?), *n*-$C_{16:1}$ (7), *n*-$C_{16:1}$ (5), *n*-$C_{18:1}$ (11) and *n*-$C_{18:1}$ (7), respectively (Table 2, Fig.4). *n*-$C_{16:1}$ (8?) is no ECL datum in Christie (1989), and we assumed based on retention time and mass spectrum.

The major fatty acids (>10%) were short-chain *n*-alkanoic acids (*n*-C_{12}, *n*-C_{14} and/or *n*-C_{16}) and *n*-alkenoic acids (*n*-$C_{16:1}$ (9), *n*-$C_{16:1}$ (8?) and/or *n*-$C_{18:1}$ (7), Table 2). The most predominant fatty acid was *n*-C_{16} alkanoic acid in all Kuroyu hot springs. Fatty acid compositions revealed that short-chain *n*-alkanoic acids, long-chain *n*-alkanoic acids, branched acids and *n*-alkenoic acids were 38.07–73.65% with an average of 62.05±12.38%, 2.30–6.17% with an average of 4.43±1.33%, 1.93–5.92% with an average of 3.67±1.40% and 16.89–52.89% with an average of 29.84±12.64%, respectively (Table 2). Short-chain *n*-alkanoic acids were most dominant fatty acids in all Kuroyu hot springs. Unexpectedly, *n*-alkenoic acids are abundant in YK01 (52.89%), YK05 (49.18%), YK010 (36.02%) and YK04 (31.19%) samples in decreasing order. These fatty acid compositions are similar to those in Kuroyu hot springs from Ohta-ku area in Tokyo (Matsumoto *et al.* (2020).

Cholesta-5,22-dien-3β-ol, cholesterol, cholestanol, brassicasterol, 24-methyl-cholesterol, 24-methylcholestanol, 24-ethylcholesterol and 24-ethylcholestanol were found in kuroyu samples from the Yokohama-Kawasaki area (Table 3). The most abundant sterol was cholesterol in all Kuroyu hot springs (31.0–80.0%) and thus C_{27} sterols were most abundant in all Kuroyu hot springs (41.5–88.0% with an average of 66.1±12.9%, Table 3).

3.4.2 Fatty acids in Py-TMAH-Me products of humic acid

Normal-alkanoic acids ranging in carbon-chain length from C_7 to C_{34} with a predominance of even-carbon numbers were found in Py-TMAH-Me products of humic acid, together with branched

Table 3 Sterols found in ethyl acetate extracts of Kuroyu hot springs from the Yokohama-Kawasaki area

Sample No,	YK01	YK02	YK03	YK04	YK05	YK06	YK07	YK08	YK09	YK10	Average±SD*
Composition （%）											
Sternol											
Cholesta-5,22-dien-3β-ol (27)	3.5	1.8	3.4	2.2	2.8	2.8	7.3	8.0	4.5	2.2	
Cholesterol (27)	31.0	53.3	51.7	49.5	50.9	65.5	69.5	80.0	73.7	72.2	
Brassicasterol(28)	16.3	14.5	8.6	13.3	13.2	19.3	0.0	4.0	8.4	10.0	
24-Methylcholsterol (28)	4.3	3.2	1.7	3.9	6.6	2.1	12.2	0.0	2.2	2.2	
Stigmasterol (29)	17.1	13.7	13.8	12.5	0.0	5.5	3.7	0.0	7.8	6.7	
24-Ethylcholesterol (29)	10.0	3.8	5.2	4.4	8.5	4.8	0.0	8.0	1.1	1.1	
Stanol											
Cholestanol (27)	7.0	6.1	6.9	6.9	8.5	0.0	3.7	0.0	2.2	5.6	
24-Methylcholestanol (28)	5.4	3.6	5.2	3.3	9.4	0.0	3.7	0.0	0.0	0.0	
24-Ethylcholesterol (29)	5.4	0.0	3.4	3.9	0.0	0.0	0.0	0.0	0.0	0.0	
Total （%）	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
C_{27}-C_{29} sterol （%）											
C_{27}-sterol	41.5	61.2	62.1	58.7	62.3	68.3	80.4	88.0	80.5	80.0	66.1±12.9
C_{28}-sterol	26.0	21.3	15.5	20.5	29.2	21.4	15.9	4.0	10.6	12.2	19.2±6.2
C_{29}-sterol	32.5	17.5	22.4	20.8	8.5	10.3	3.7	8.0	8.9	7.8	14.7±9.2
Total （%）	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	

*Standard deviation. YK08 sample was excluded for the calculation of average and SD.

(*iso*- and *anteiso*-$C_{13\text{-}17}$), and alkenoic acids (four peaks $C_{16:1}$ and two peaks $C_{18:1}$), although no polyunsaturated fatty acids were detected in Yokohama-Kawasaki samples (Table 4) as in the case of ethyl acetate extract, Table 2). Interestingly, the pattern of alkenoic acids in Py-TMAH-Me product of humic acid is similar to those of ethyl acetate extract (Table 2). Four $C_{16:1}$ peaks and two $C_{18:1}$ peaks in the gas chromatogram were identified as in the case of ethyl acetate extract, as *n*-$C_{16:1}$ (9), *n*-$C_{16:1}$ (8?), *n*-$C_{16:1}$ (7), *n*-$C_{16:1}$ (5), *n*-$C_{18:1}$ (11) and *n*-$C_{18:1}$ (7) (Fig. 4, Table 4).

The major fatty acids (>10%) were *n*-C_{14}, *n*-C_{16}, *n*-$C_{16:1}$ (9), *n*-$C_{16:1}$ (8?), n-$C_{16:1}$ (7), and/or *n*-$C_{16:1}$ (5) (Table 4). The most predominant fatty acid was *n*-C_{16}, *n*-$C_{16:1}$ (9), or *n*-$C_{16:1}$ (8?). Fatty acid compositions revealed that short-chain *n*-alkanoic acids (*n*-C_{12}-*n*-C_{19}), long-chain *n*-alkanoic acids (*n*-C_{20}-*n*-C_{34}), branched acids (*n*-C_{13}-*n*-C_{17}) and *n*-alkenoic acids (*n*-$C_{16:1}$ and *n*-$C_{18:1}$) were 20.50–49.34% with an average of 30.24±10.18%, 2.63–10.80% with an average of 5.24±2.49%, 2.33–5.62% with an average of 3.63±1.00% and 34.47–72.27% with an average of56.23±12.37%, respectively (Table 4). Very short-chain *n*-alkanoic acids (*n*-C_7-*n*-C_{11}) were present in Py-TMAH-Me products of humic acid from all Kuroyu hot springs. Short-chain *n*-alkanoic acids were most dominant in YK09 sample, while interestingly *n*-alkenoic acids were most abundant in YK01, YK02, YK03, YK04, YK05, YK06, YK07, YK08 and YK10 samples (Table 4). This result suggests strongly that *n*-alkenoic acids are major structural components of humic acid.

3.5 Sources of organic components

Fatty acids are widely distributed in every organism except for Archaea. Short-chain *n*-alkanoic acids (*n*-C_{10}-*n*-C_{19}) are major components of algae, and are biomarker of algae including plankton, while long-chain *n*-alkanoic acids (*n*-C_{20}-*n*-C_{34}) are abundant in the waxes of vascular plants, and are biomarker of vascular plants (Matsumoto and Watanuki, 1992; Matsumoto, 2014). Branched acids (*iso* and *anteiso*-C_{13}-C_{17}) are biomarkers of bacteria (O'Leary, 1982 ; Reddy *et al.*,

Table 4　Fatty acids found in Py-TMAH-Me products of humic acid in Kuroyu hot springs from the Yokohama-Kawasaki area

Sample No.	YK01	YK02	YK03	YK04	YK05	YK06	YK07	YK08	YK09	YK10	Average±SD
n-Short (%)											
7	0.65	0.40	1.78	0.40	0.89	0.76	0.67	0.69	1.20	0.87	
8	0.88	0.76	2.89	0.71	1.66	1.23	0.96	1.41	2.11	1.61	
9	0.66	0.46	2.27	0.52	1.29	1.13	0.87	1.89	2.70	1.19	
10	0.55	0.36	1.60	0.35	0.95	0.68	0.60	0.86	1.20	0.79	
11	0.33	0.19	0.92	0.17	0.44	0.25	0.32	0.18	0.34	0.33	
12	17.03	1.33	2.44	1.53	2.08	4.78	2.02	4.29	3.36	3.23	
13	0.52	0.26	1.07	0.23	0.46	0.35	0.38	0.34	0.44	0.40	
14	9.68	9.33	6.00	9.64	7.90	10.32	6.35	8.53	7.59	7.22	
15	1.97	1.07	2.22	1.17	1.71	2.27	1.67	3.00	2.98	1.81	
16	11.29	7.92	10.22	6.94	9.98	15.66	12.50	18.43	30.19	12.66	
17	0.63	0.21	0.82	0.23	0.56	0.55	0.58	0.77	0.92	0.56	
18	1.52	0.65	2.67	0.77	1.87	2.39	1.63	3.09	3.85	1.61	
Total (7-11, %)	3.07	2.17	9.46	2.14	5.24	4.05	3.42	5.03	7.56	4.79	4.65 ± 2.47
Total (12-18, %)	42.64	20.77	25.44	20.50	24.56	36.32	25.12	38.44	49.34	27.50	30.24 ± 10.18
n-Long (%)											
20	0.36	0.20	0.69	0.19	0.35	0.33	0.35	0.41	0.41	0.34	
21	0.18	0.09	0.45	0.09	0.26	0.16	0.18	0.11	0.18	0.14	
22	0.59	0.32	1.22	0.32	0.69	0.45	0.49	0.47	0.52	0.67	
23	0.24	0.13	0.49	0.11	0.21	0.22	0.19	0.19	0.22	0.20	
24	0.97	0.44	1.64	0.35	0.91	0.75	0.62	0.82	0.85	0.93	
25	0.27	0.10	0.43	0.08	0.18	0.22	0.18	0.23	0.26	0.19	
26	0.52	0.25	0.97	0.17	0.48	0.39	0.31	0.34	0.44	0.59	
27	0.16	0.10	0.39	0.07	0.14	0.16	0.13	0.09	0.10	0.18	
28	0.46	0.33	1.02	0.22	0.61	0.32	0.20	0.21	0.33	0.81	
29	0.16	0.14	0.43	0.13	0.19	0.14	0.11	0.05	0.07	0.29	
30	0.40	0.41	1.26	0.28	0.06	0.39	0.19	0.13	0.30	1.04	
31	0.07	0.15	0.32	0.10	0.21	0.17	0.17	0.03	0.08	0.29	
32	0.33	0.37	0.89	0.29	0.54	0.37	0.22	0.16	0.32	0.93	
33	0.14	0.12	0.22	0.06	0.09	0.20	0.10	0.03	0.10	0.24	
34	0.20	0.19	0.37	0.17	0.29	0.33	0.14	0.19	0.38	0.54	
Total *n*-long (%)	5.04	3.36	10.80	2.63	5.23	4.61	3.58	3.45	4.55	7.38	5.24 ± 2.49
Branched*											
i12	0.13	0.03	0.35	0.05	0.25	0.06	0.09	0.09	0.11	0.08	
i13	0.34	0.16	0.20	0.09	0.21	0.08	0.22	0.09	0.10	0.07	
i14	1.31	0.50	0.62	0.90	1.14	0.78	0.99	0.99	0.91	0.53	
i15	1.70	1.48	0.59	0.50	0.60	1.31	1.21	0.60	0.72	0.51	
i16	0.75	0.25	0.44	0.26	0.46	0.77	0.61	0.69	0.77	0.42	
i17	0.14	0.13	0.14	0.05	0.06	0.06	0.20	0.13	0.29	0.06	
a13	0.15	0.08	0.18	0.09	0.13	0.08	0.08	0.12	0.14	0.09	
a15	1.04	0.90	0.64	0.49	0.79	0.88	0.46	0.69	0.77	0.51	
a17	0.18	0.12	0.18	0.08	0.13	0.15	0.16	0.26	0.38	0.13	
Total branched (%)	5.62	3.63	3.01	2.45	3.53	4.10	3.92	3.55	4.09	2.33	3.63 ± 1.00
n-Alkenoic (%)#											
16:1(9)	14.16	24.80	9.00	30.82	18.70	17.80	19.42	18.94	6.39	16.37	
16:1(8?)	12.46	16.54	18.73	16.46	16.22	14.40	20.16	10.93	18.12	18.87	
16:1(7)	4.82	10.01	3.67	4.79	6.45	4.93	5.34	2.23	1.67	5.87	
16:1(5)	7.37	16.98	6.33	16.96	9.71	8.03	13.33	6.13	1.96	9.29	
18:1(11)	2.83	0.87	5.51	1.50	3.91	2.87	2.38	7.59	4.95	2.66	
18:1(7)	1.99	0.87	8.06	1.75	6.45	2.89	3.33	3.72	1.37	4.94	
Total *n*-alkenoic (%)	43.64	70.08	51.30	72.27	61.44	50.92	63.96	49.53	34.47	58.00	56.23 ± 12.37
Total(%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	

*Standard deviation. YK08 sample was excluded for the calculation of average and SD.

#i and a are *iso*- and *anteiso*-acids, respectively.

$Carbon chain length: number of unsaturation (double bond position).

2002, 2003a, 2003b). Polyunsaturated fatty acids are generally not produced by bacteria (O'Learly, 1982; Reddy *et al.*, 2002, 2003a, 2003b). Very short-chain *n*-alkanoic acids (*n*-C_7-*n*-C_8) are not common in natural products except for the milk of various mammals (Curro *et al.*, 2019).

The abundance of short-chain *n*-alkanoic acids with small amounts of long-chain *n*-alkanoic acids and branched acids revealed that the major sources of fatty acids are algae with small contribution of vascular plants and bacteria. It is similar to those of fatty acids of Py-TMAH-Me products of humic acid (Table 4). Humic acid is, therefore, mainly derived from marine algae. Unusual abundance of normal mono-unsaturated fatty acid in ethyl acetate extracts and Py-TMAH-Me products of humic acid can be explained by the degradation loss of polyunsaturated fatty acids in the sedimentary environments, since mono- and polyunsaturated fatty acids ($C_{16:1}$, $C_{16:2}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:5}$, $C_{22:6}$, etc.) are widely distributed in algae including phytoplankton and zooplankton in lacustrine and marine environments (Weete, 1976; Kawamura *et al.*, 1980; Kawamura and Ishiwatari, 1981).

Wakeham (2000) studied particulate organic carbon and lipid fluxes in the water column and surface sediments in 3 sites of the equatorial Pacific Ocean (9°N, 5°N and equator along 140°W). Estimates of lipid production by plankton in the euphotic zone range from 30–290 mg lipid/m^2d. Fluxes of lipids decreased quickly in the water column, resulting in delivery rates of lipids to sediments that were 5–6 orders of magnitude reduced (0.00004–0.032 mg lipid/m^2d, Wakeham, 2000). A diagenesis proceeded down the water column that polyunsaturated fatty acids decreased and disappeared in the water column and surface sediments, but monounsaturated fatty acids and branched acids increased (Wakeham, 2000). Thus, absence of polyunsaturated acids and abundance of *n*-mono-alkenoic acids in Kuroyu hot springs are attributed to the preferential degradation loss such as by oxidation of polyunsaturated acids in the sedimentary environment. Very little is known on the double bond position of *n*-alkenoic acids in natural environments. Further geochemical studies on double bond position of *n*-alkenoic acids in relation to their characteristics and source organisms are strongly required.

Takano *et al.* (2020) studied ratios of carbon, nitrogen and phosphorous in humus (humic and fulvic acid) in same samples of our Yokohama-Kawasaki area. C/N molar ratio of humic acid and fulvic acid ranged from 3.7 to 24 and from 5.2 to 21, respectively. These C/N molar ratios values suggest that humus is originated mainly from marine algae with contribution of terrestrial vascular plants to the high values.

Sterols (stenols and stanols) are widely distributed in lacustrine and marine environments. Cholesterol is a typical sterol of algae including phytoplankton and is biomarker of their organisms (Matsumoto *et al.*, 1982, 2006; Volkman *et al.*, 1998). 24-methylcholesterol is often abundant in certain diatoms and is a biomarker of diatoms (Rampen *et al.*, 2010). C_{29} sterol (24-ethylcholesterol and 24-ethylcholesta-5,22-dien-3*β*-ol) are predominant in vascular plants, and generally accepted as their biomarker (Matsumoto *et al.* 1982, 2006).

Sterol composition showed that cholesterol is most predominant sterol in all Kuroyu hot springs (Table 3). They are derived from algae including plankton with some contribution of diatom and vascular plants.

Stanols are found in certain microalgae (dinoflagellates, diatoms and raphidphyte (Volkman *et al.* 1998) and are also formed by bacterial reduction of stenols (Nishimura 1982). Stanols are increased in the water column and sediments in the Pacific Ocean (Wakeham, 2000). Stanols are, therefore, derived from microalgae in the sedimentary environments in addition to microbial reduction of stenols.

4. Conclusion

Geochemical studies on Kuroyu hot springs in the Yokohama-Kawasaki area of Kanagawa Prefecture were carried out to elucidate their characteristics, especially focused on organic components including humic acid, together with source organisms and sedimentary environments.

(1) Kuroyu hot spring qualities are mainly sodium-hydrogen carbonate or sodium-hydrogen carbonate/chloride. They are similar to those in Ohta-ku area in Tokyo of Minami-Kanto gas field.

(2) The inverse correlation between Log-transparency and humic acid contents showed that color levels of Kuroyu hot springs are attributed to humic acid contents.

(3) Saturated fatty acids are mainly composed of short-chain *n*-alkanoic acid (C_{12}-C_{18}) with small amounts of long-chain *n*-alkanoic acids (C_{20}-C_{34}) and branched acids (*iso*- and *anteiso*-C_{12}-C_{17}) in ethyl acetate extracts and Py-TMAH-Me products of humic acid.

(4) Unusually, *n*-alkenoic acids (*n*-$C_{16:1}$ (9), *n*-$C_{16:1}$ (8?), *n*-$C_{16:1}$ (7), *n*-$C_{16:1}$ (5), *n*-$C_{18:1}$ (11) and *n*-$C_{18:1}$ (7)) are abundant in ethyl acetate extracts and Py-TMAH-Me products of humic acids.

(5) Major sterols in ethyl acetate extracts were all cholesterol with small amounts of 24-methylcholesterol and 24-ethylcholesterol and others.

(6) Organic components in Kuroyu hot springs are mainly originated from marine algae with some contribution of vascular plants and bacteria. Unusual abundance of normal mono-unsaturated fatty acids can be explained by the degradation loss of polyunsaturated fatty acids in the water column and sedimentary environments.

(7) Well depths (70–200 m) imply that sedimentary environments are Tsurukawa Formation of Kazusa Group forms in the Early Pleistocene (2.58–0.77 Ma) with marine depths of 25–50 m, while well depth of 1,500 m suggests that sedimentary environment is Ikego Formation of Miura Group forms in Late Pliocene (3.60–2.58 Ma) marine deposit of bathylal zone environment.

Acknowledgments

The authors are greatly appreciated to the owners of Kuroyu hot springs (Sentous) for their kind supports of the sampling and offering hot spring analysis data sheet and well depth information. We are deeply grateful to the owners of Kuroyu hot springs in the Yokohama-Kawasaki area for their kind cooperation in sampling. We thank Asuka Ono of Otsuma Women's University for her kind assistance in sampling. We thank two anonymous reviewers for their useful and valuable comments.

References

Aoyanagi, N., Ichihashi, D. and Uchino, E. (2005): Humic acid concentrations of hot springs in Hokkaido. *Abstract of the 58th Annual Meeting of the Japanese Society of Hot Spring Sciences*, 50 (in Japanese).

Baba, K. (2015) The molluscan fossil assemblages of the Lower Pleistocene Kazusa Group of the Western Flank of the Kwanto Plain and environmental change. The Annals Gifu Shotoku University, *Faculty of Education*, **54**, 65–87.

Christie, W.W. (ed., 1989): Chapter 5 Gas chromatographic analysis of fatty acid derivatives. In Gas Chromatography and Lipids A Practical Guide, pp. 85–128, The Oily Press, Ayr, Scotland.

Currò, S., Manuelian, C.L., Marchi, M.D., Claps, S., Rufrano, D. and Neglia, G. (2019): Effects of breed and stage of lactation on milk fatty acid composition of Italian goat breeds. *Animals*, **9**, 764 (doi: 10.3390/ani9100764).

Daita, Y. and Odawara, J. (2014): Incidental gas from hot spring waters in Kanagawa Prefecture (Part IV). *Bull. Hot Springs Res. Inst. Kanagawa Pref.*, **46**, 34–42 (in Japanese).

Daita, Y., Kikugawa, J., Odawara, K., Itadera, K., Mannen, K. and Harada A. (2007): Measures of Kanagawa Prefectural government to prevent explosion caused by methane gas dissolved in hot spring waters. *Bull. Hot Springs Res. Inst. Kanagawa Pref.*, **39**, 89–98 (in Japanese).

Eto, T. (1986): Stratigraphy of the Miura and Kazusa Groups in the Miura Peniunsula, Japan. *Science Repots of the Yokohama National University*, Sec. II, No. 33, 107–132 (in Japanese with English abstract).

Geological Survey of Japan (1976): Distribution of oil and gas fields in Japan (including offshore areas, Second Edition), edited by K. Yazaki, Geological Map Series, No. 9, 1 : 2,000,000 (in Japanese).

Imahashi, M. (2015): Easy-to-understand hot spring chemistry. ④ Brown hot spring. *Hot Spring (Onsen)*, 2015 Winter Issue, 28–29.

International Commission on Stratigraphy (2024): International Chronostratigraphic Chart. IUGS, www.stratigraphy.org.

Ishiwata, S. (2004): The Late Pleistocene-Holocene deposits and the sedimentary environment in the northern coastal area of Tokyo Bay, Central Japan. *Quaternary Research*, **43**, 297–310 (in Japanese with English abstract).

Ishiwatari, R. (2008): Humic substances in nature. In Humic Substances in Natural Environment —Their Characteristics and Research (ed. Ishiwatari, R.), pp. 2–9, Sankyo Pub., Tokyo (in Japanese).

Kanroji, Y. (1999a): Hot spring old story Hot springs in Tokyo (1) History of the development of hot spring resources in 23 wards. *Nihon Onsen Kanrishi Gakkai-shi (Journal of the Japan Hot Spring Managers Association),* **22**, 1–10 (in Japanese).

Kanroji, Y. (1999b): Hot spring old story Hot springs in Tokyo (2) Current status of hot spring resources in 23 wards—Especially chemical components—. *Nihon Onsen Kanrishi Gakkai-shi (Journal of the Japan Hot Spring Managers Association)*, **22**, 11–20 (in Japanese).

Kanroji, Y. (2010): Colored substances of hot spring water in plain area—Non-volcanic hot spring water—. *Nihon Onsen Kanrishi Gakkai-shi (Journal of the Japan Hot Spring Managers Association)*, **43**, 18–24 (in Japanese).

Kawamura, K., Ishiwatari, R. and Yamazaki, M. (1980): Identification of polyunsaturated fatty acids in surface lacustrine sediments. *Chem. Geol.*, **28**, 31–39.

Kawamura, K. and Ishiwatari, R. (1981): Polyunsaturated fatty acids in a lacustrine sediment as a possible indicator of paleoclimate. *Geochim. Cosmochim. Acta*, **45**, 149–155.

Kikuchi, T. (1980): Paleo Tokyo Bay. *Urban Kubota*, **18**, 16–21 (in Japanese).

Mashiko, Y., Kanroji, Y., Sato, K. and Adachihara, A. (1959): Geochemical studies on mineral springs. *J. Balneolog. Soc. Jap. (*now *J. Hot Spring Sci.)*, **10**, 87–91 (in Japanese).

Matsumoto G.I. (2014): Organic geochemical studies of lipid biomarkers in inland hydrothermal environments (hot springs). *J. Hot Spring Sci.*, **64**, 209–242 (in Japanese with English abstract).

Matsumoto, G.I., Akutsu, Y. and Takamatsu, N. (2006): Features and sources of organic components in surface sediments of Lake Nishi-inbanuma in Chiba Prefecture, Japan. *Jap. J. Limnol.*, **67**, 1-11 (in Japanese with English abstract).

Matsumoto, G.I., Fujimura, C., Minoura, K., Takamatsu, N., Takemura, T., Hayashi, S., Shichi, K. and Kawai, T. (2003): Paleoenvironmental changes in the Eurasian continental interior during the last 12 million years derived from organic components in sediment cores (BDP-96 and BDP-98) from Lake Baikal. In Long Continental Records from Lake Baikal (ed. Kashiwaya, K.), pp. 75–94, Springer-Verlag, Tokyo.

Matsumoto, G.I. and Ohmiya, M. (2019): Analysis of fatty acids in humic acids of Kuroyu hot springs from the Kamata area in Tokyo by pyrolysis TMAH methylation GC/MS method. *J. Hot Spring Sci.*, **68**, 230–239 (in Japanese with English abstract).

Matsumoto, G.I., Shimizu, R. and Nagura M. (2020): Geochemical characteristics of Kuroyu hot springs in the Ohta-ku area of Tokyo. *J. Hot Spring Sci.*, **70**, 150–169.

Matsumoto, G., Torii, T. and Hanya, T. (1979): Distribution of organic constituents in lake waters and sediments of the McMurdo Sound region in the Antarctic. *Mem. Natl. Inst. Polar Res. Spec. Iss.*, **13**, 103-120.

Matsumoto, G., Torii, T. and Hanya, T. (1982): High abundance of algal 24-ethylcholesterol in Antarctic lake sediment. *Nature*, **299**, 52–54.

Matsumoto, G.I. and Watanuki, K. (1992): Organic geochemical features of an extremely acid crater lake (Yugama) of Kusatsu-Shirane Volcano in Japan. *Geochem. J.*, **26**, 117-136.

Muramatsu, Y., Okazaki, K., Oshiro, E. and Yasumoro, M. (2008): Hydrochemistry and genesis of non-volcanic hot springs from the central Kanto Plain, central Japan. *Chikasui Gakkaishi (J. Groundwater Hydrology)*, **50**, 145–162 (in Japanese with English abstract).

Natural Environment Bureau, Ministry of the Environment (2014): Determination of humus. Guidelines for Mineral Spring Analysis (Heisei 26 Revised Edition), pp. 145–146, Ministry of the Environment, Tokyo (in Japanese).

Nishimura, M. (1982): 5*β*-isomers of stanols and stanones as potential markers of sedimentary organic quality and depositional paleoenvironments. *Geochim. Cosmochim. Acta*, **46**, 423–432.

O'Leary, W.M. (1982): Lipoidal contents of specific microorganisms. CRC Handbook of Microbiology, 2nd Ed., Vol. IV. Microbial composition: Carbohydrates, Lipids, and Minerals (eds. Laskin A.I. and Lechevalier, H.A.), pp. 391–434, CRC Press, Boca Raton, FL.

Ozawa, K. and Eto, T. (2005): Geology of deep hot spring wells and underground geological structure in the central and eastern parts of Kanagawa Prefecture. *Bull. Hot Springs Res. Inst. Kanagawa Pref.*, **37**, 15–38 (in Japanese).

Petroleum Technology Association (ed. 1983): Distribution range of soluble natural gas hot spring deposits. Petroleum Mining Handbook, p. 177, The Japanese Association for Petroleum Technology, Tokyo (in Japanese).

Rampen, S.W, Abbas, B.A., Schouten, S. and Sinninghe Damaste, J.S. (2010): A comprehensive study of sterols in marine diatoms (Bacillariophyta): Implications for their use as tracers for diatom productivity. *Limnol. Oceanogr.*, **55**, 91-105.

Reddy, G.S.N., Prakash, J.S.S., Vairamani, M., Prabhakar, S., Matsumoto, G.I. and Shivaji, S. (2002): *Planococcus antarcticus* and *Planococcus psychrophilus* spp.nov.isolated from cyanobacterial mat samples collected from ponds in Antarctica. *Extremophiles*, **6**, 253-261.

Reddy, G.S.N., Prakash, J.S.S., Prabahar, V., Matsumoto, G.I., Stackebrandt, E. and Shivaji, S. (2003a): *Kocuria Polaris* sp. nov., an orange-pigmented psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample. *Int. J. Syst. Evol. Microbiol.*, **53**, 183-187.

Reddy, G.S.N., Prakash, J.S.S., Srinivas, R., Matsumoto, G.I. and Shivaji, S. (2003b): *Leifsonia rubra* sp. nov. and *Leifsonia aurea* sp. nov., psychrophiles from a pond in Antarctica. *Int. J. Syst. Evol. Microbial.*, **53**, 977-984.

Seki, Y., Nakajima, T., Kamioka, H., Kanai, Y., Manaka, M. and Tsukimura, K. (2004): Discharged water from deep wells in the eastern Kanto region—the relationship between water quality and underground geology—. *J. Balneolog. Soc. Jap. (*now *J. Hot Spring Sci.)*, **54**, 1-24 (in Japanese with English abstract).

Sekimoto, K., Yoshikawa, M., Anma, K., Shimizu, K. and Endo, K. (2009): Microfossil analysis and paleoenvironmental changes of Kazusa and Shimousa Groups from cores in the Port of Tokyo, central Japan. *Bulletin of the Institute of Natural Science, Faculty of Letters and Science, Nihon University*, **44**, 139-148 (in Japanese with English abstract).

Suzuki, R. (1979): Fluoride in alkaline mineral springs. *Chikyu Kagaku (Geochemistry)*, **13**, 25-31 (in Japanese with English abstract).

Takamatsu, N., Shimodaira, K., Imahashi, M. and Yoshioka, R. (1981): A study on the chemical composition of ground waters from granitic areas. *Chikyu Kagaku (Geochemistry)*, **15**, 69-76 (in Japanese with English abstract).

Takano, K., Matsumoto, G.I., and Uchino, E. (2020) Characteristics of humus in Kuroyu (blackish colored spring) pumped up in the Yokohama and Kawasaki area, Kanagawa, Japan, by ratios of carbon to nitrogen or to phosphorus. *J. Hot Spring Sci.*, **70**, 137-149 (in Japanese with English abstract).

Takano, K., Uchino, E. and Aoyanagi, N. (2016): Analysis and characteristics of humic acid in spring water from central Hokkaido, Japan. *Jap. J. Limnol.*, **77**, 167-174 (in Japanese with English abstract).

Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L. and Gelin, F. (1998): Microalgal biomarkers. A review of recent research developments. *Org. Geochem.*, **29**, 1163-1179.

Wakeham, S.G. (2000): Organic matter preservation in the ocean: Lipid behavior from plankton to sediments. In Chemical Processes in Marine Environments (eds. Gianguzza, A., Pelizzetti, E. and Sammartano, S.), pp. 127-139, Springer-Verlag, Berlin.

Weete, J.D. (1976): Algal and fungal waxes. In Chemistry and Biochemistry of Natural Waxes (ed. Kolattukudy, P.E.), pp. 349-417, Elsevier, Amsterdam.

Yamamoto, S., Yoshioka, H. and Ishiwatari, R. (2007): Pyrolysis- and chemical degradation-GC/MS analyses of environmental kerogen and humic substances and their applications to geochemistry. *Bunseki Kagaku (Analytical Chemistry)* , **56**, 72-87 (in Japanese with English abstract).

A part of the paper was presented orally at the 72nd Annual Meeting of the Japanese Society of the Hot Spring Sciences, and at the 66th Annual Meeting of the Geochemical Society of Japan.