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原　　著

Geochemical Characteristics of Kuroyu Hot Springs in the Ohta-ku Area of Tokyo

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東京都大田区地域における黒湯の地球化学的特徴

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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．本研究では東京都大田区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．これらの泉質の生成には，花崗岩の寄与レベルやモンモリロナイトのイオン交換反応が推定される．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．驚くべきことに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 tetramethylammoniumhydroxide 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，浅海の古東京湾（下総層群）および半深海の陸棚斜面（上総層群）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，一不飽和酸脂肪酸が残存していると考えられる．堆積年代は下総層群および上総層群上部の 0.5-1 Ma と考えられる．

キーワード：東京都大田区地域，黒湯，バイオマーカー，フミン酸，熱分解 TMAH メチル化，下総層群・上総層群

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Abstract

Geochemical studies on Kuroyu hot springs in Ohta-ku area of Tokyo 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 are mainly sodium-hydrogen carbonate or sodium-hydrogen carbonate/chloride. These hot springs may be formed by contribution levels of granitic rocks and ion exchange reaction of montmorillonite. 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-tetramethylammniumhydroxide-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 are ascribed to the degradation loss in the sedimentary environments. Well depths (30–120 m) showed that sedimentary environments are shallow Paleo Tokyo Bay of Shimousa Group and marine land shelf slope of upper Kazusa Group in ages 0.5–1 Ma.

Key words : Ohta-ku area in Tokyo, 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). These colored hot springs containing humic substances are associated with the area of water-soluble natural gas deposit (Fig. 1, Petroleum Technology Association,1983 ; Kanroji, 2010). 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 in humic acid by pyrolysis-tetramethylammoniumhydroxide methylation gas chromatography/mass spectrometry (Py-TMAH-Me-GC/MS) in Kuroyu hot springs from Kamata area in Tokyo (Matsumoto and Ohmiya, 2019). 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 products, 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).

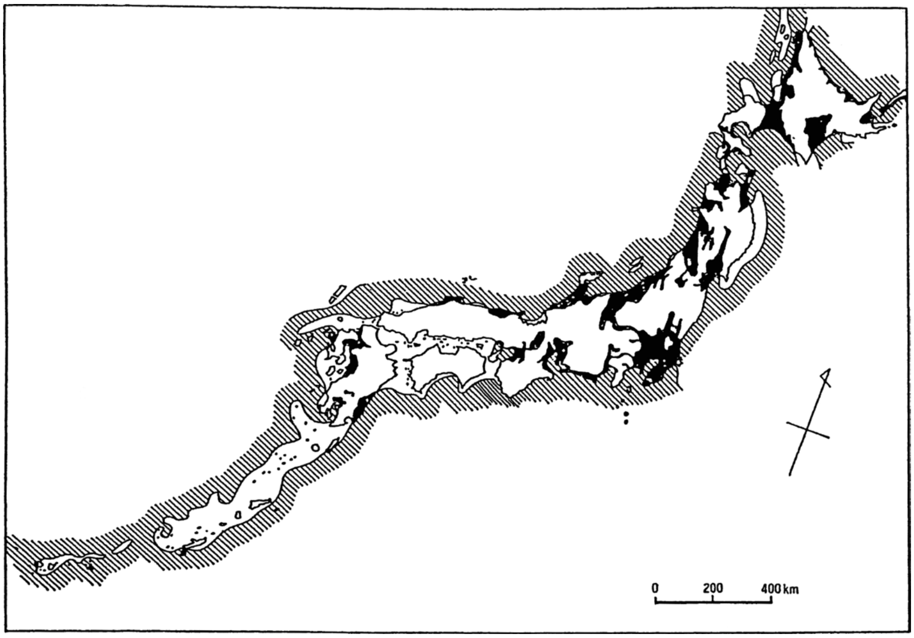

Fig. 1 Distribution of water soluble natural gas deposits in Japan (Petroleum Technology Association, 1983 ; Kanroji, 2010).

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 (Sentou) from the Ohta-ku area in Tokyo 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 Koto-ku, Edogawa-ku and the south region of Tokyo wards area (Fig. 2, Tokyo Metropolitan Bureau of Economics, 1955 ; Kawashima *et al*., 1996). In August 25 and 26, 2016, Kuroyu hot springs (OT01-OT10) were sampled from hot water supply port of Kuroyu hot springs in 2 L polyethylene bottles from the Ohta-ku area in Tokyo (Fig. 2, Table 1). The Kuroyu hot springs were kept in a refrigerator until analyses.

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 Mineral Spring Analysis Guidelines (Natural Environment Bureau, Ministry of the Environment, 2014). Kuroyu hot springs were fractionated in 100 mL glass bottles with screw caps, and added 2 mL concentrated hydrochloric acid to precipitate humic acid, and then to stand for overnight at room temperatures. Humic

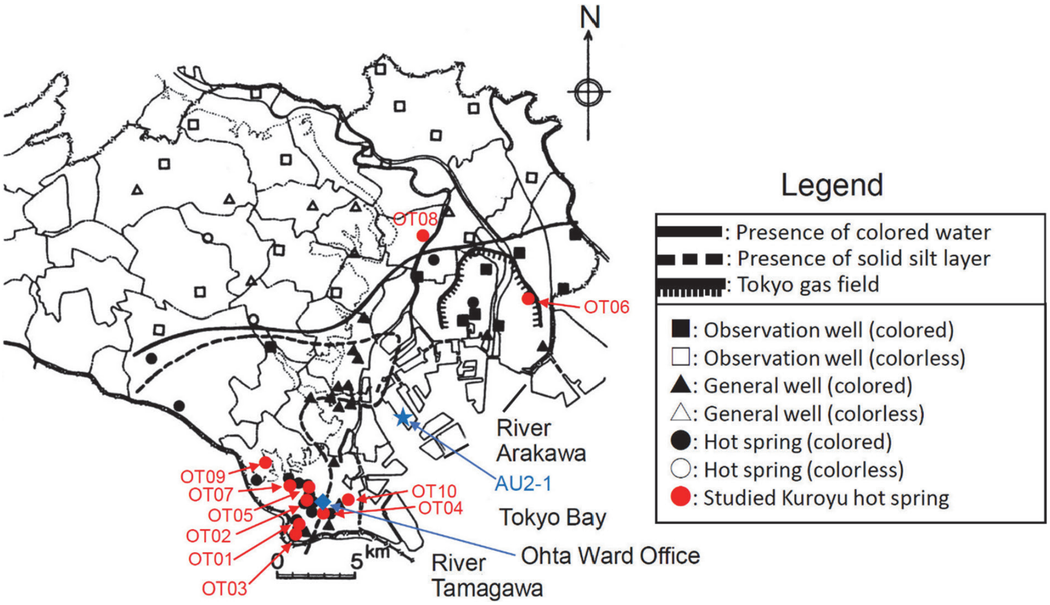

Fig. 2　Sampling sites of Kuroyu hot springs (Sentou) and AU2-1 sediment core site in the Ohta-ku area of Tokyo. Kuroyu sampling sites were added for figure of colored water distribution by Kawashima *et al*., 1996). AU2-1 sediment core site (Tokyo Metropolitan Bureau of Port and Harbor,1993 ; Sekimoto *et al*., 2009).

Table 1　Physico-chemical characteristics of Kuroyu hot springs in the Ohta-ku area of Tokyo

Sample No.	Sampling date	Water temp. (℃)*	pH*	Electric cond. (mS/m)*	Total dissoved matter (g/kg)*	Well depth (m)*	Trans-parency (cm)	Humic acid (mg/L)	Hot spring quality*
OT01	25/08/2016	17.5	8.3	121.7	2.269	90	1.8	325	Na-HCO_3
OT02	25/08/2016	17.4	8.4	248.0	3.115	50	3.0	281	Na-HCO_3・Cl
OT03	26/08/2016	16.1	8.4	142.2	2.212	50-60	3.7	211	Na-HCO_3・Cl
OT04	25/08/2016	17.5	7.68	136.0	2.335	100	7.5	159	Na-HCO_3・Cl
OT05	25/08/2016	18.0	8.4	200.0	1.869	114	4.8	189	Na-HCO_3・Cl
OT06	25/08/2016	17.9	8.5	65.4	0.570	100	28.0	33.4	Na-HCO_3・Cl
OT07	25/08/2016	18.7	7.7	147.0	1.106	30, 100	>30	11.3	Na-Cl・HCO_3
OT08	26/08/2016	18.0	7.83	72.9	0.635	120	>30	7.9	Na-HCO_3・Cl
OT09	25/08/2016	17.5	8.4	40.5	0.415	100	>30	6.3	Na-HCO_3
OT10	25/08/2016	16.6	7.6	281.0	1.716	100	>30	5.8	Na・Ca・Mg-Cl・HCO_3

*Hot spring analytical data sheet and personal communication.

acid was precipitated completely and transparent water were obtained (Fig. 3). Humic acid was collected by filtration through glass fiber paper (Whatman, GF/C pore size 0.12 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

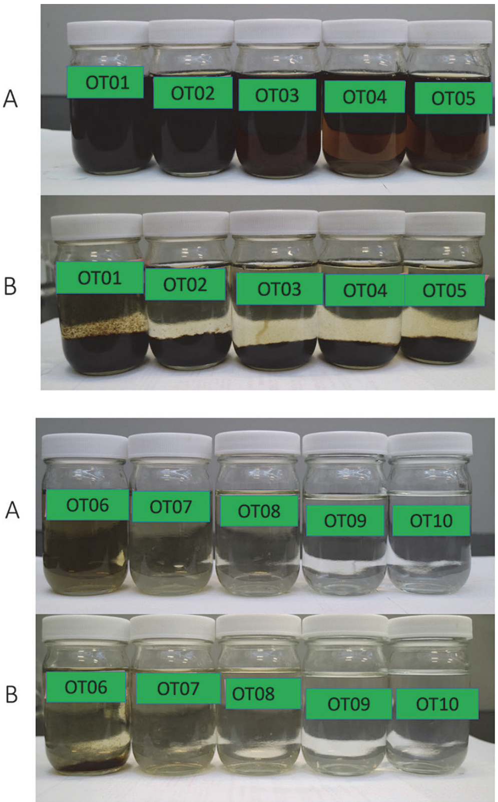

Fig. 3　Kuroyu hot springs (OT01-OT10) from the Ohta-ku area in Tokyo.
A : Before addition of hydrochloric acid.　B : Precipitation of humic acid after addition of hydrochloric acid

aspirator in a screw-in desiccator at 40℃ in a 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, selected five Kuroyu hot springs (OT01-OT05, 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 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 gas chromatograph-mass spectrometer (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 Ohta-ku area

Tokyo Metropolitan Bureau of Port and Harbor (1993) surveyed basic geology of the port of Tokyo, and taken a number of sediment cores including 400 m AU2-1 core in the northwest of Tokyo Bay (35°36′19″N, 139°47′02″E) approximately 7.8 km of the Ohta Ward Office (Fig. 2). The AU2-1 sediment core consists of Kazusa Group (Kiwada Formation, Ohtadai F., Umegase F. and Kokumoto F.), Shimousa Group (Edogawa F., Tokyo F.), Yurakucho Formation and reclaimed land layer (Fig. 4, Ishiwata, 2004 ; Sekimoto *et al.*, 2009 ; Akiyama *et al.*, 2012).

Kazusa Group in AU2-1 core from the bottom to the upward is formed in the lower, middle and upper marine land shelf slopes, respectively (Sekimoto *et al.*, 2009). Shimousa Group is formed in Paleo Tokyo Bay of shallow marine environments (Kikuchi, 1980). The sea levels are largely fluctuated with glacial and inter glacial cycles. The boundary of Kazusa and Shimousa Group is

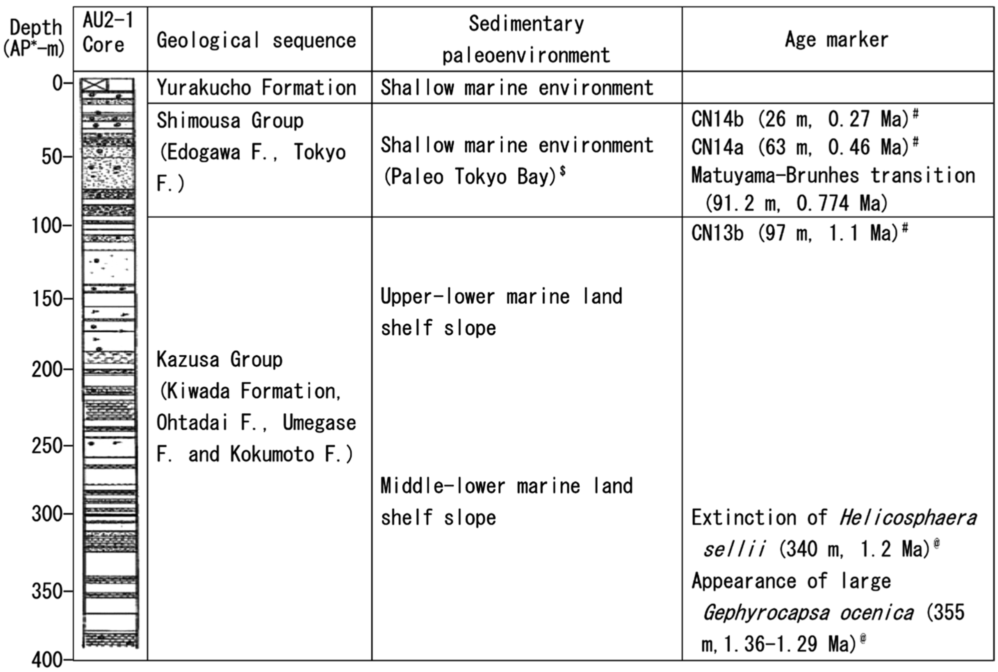

Fig. 4 Sedimentary sequence and sedimentary paleoenvironments (reorganized from Sekimoto *et al.*, 2009). ＊Arakawa Peil (Tokyo Peil-1.134 m). [$] Kikuchi (1980). [#] Tokyo Metropolitan Bureau of Port and Harbor (1993). [@] Okada and Bukry (1980).

assumed to be marine oxygen isotope stage 16 (ca. 0.65 Ma, Kikuchi, 1997, 2004 ; Fig. 4). Matuyama-Brunhes transition (0.774 Ma) was found at 91.2 m of AU2-1 core (Sekimoto *et al*., 2009). Extinction of *Helicosphaera sellii* was found at 340 m (1.2 Ma). Well depths of Kuroyu hot springs ranged from 30 to 120 m, and thus the sediment ages are expected to be ca. 0.50–1 Ma.

3.2 Hot spring quality

Table 1 shows physico-chemical properties of Kuroyu hot springs from the Ohta-ku area in Tokyo. Water temperature, pH, electric conductivity, chemical components, 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. Water temperatures ranging from 16.1 to 18.7℃ with an average of 17.5 ± 0.6 (standard deviation) showed all cold mineral springs. pH ranging from 7.6 to 8.5 with an average of 8.2 ± 0.5 showed all weak alkaline reflecting major hot spring quality of sodium-hydrogen carbonate and sodium-hydrogen carbonate/chloride (Table 1). Well depths ranged from 30 to 120 m correspond to the Shimousa Group formed in shallow marine environment of Paleo Tokyo Bay (Kikuchi, 1980) or upper Kazusa Group formed in marine land shelf slope (Sekimoto *et al*., 2009, Figs. 4).

Major cations and anions in the Kuroyu hot springs are shown in Fig. 5. Most predominant cation was Na^+ in all Kuroyu hot springs, especially OT01-OT07 samples Na^+ exceed greater than 80%. OT08-OT10 samples contain considerable amounts of Ca^{2+} (17.2–33.9%). OT10 sample contains Mg^{2+} greater than 33%. Most predominant anion of HCO_3^- is found in OT01-OT06, OT08 and OT09 samples, while Cl^- is the most predominant anion of OT10 and OT07 samples.

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). The formation processes of major cations and anions in Kuroyu hot springs in the Ohta-ku area can be

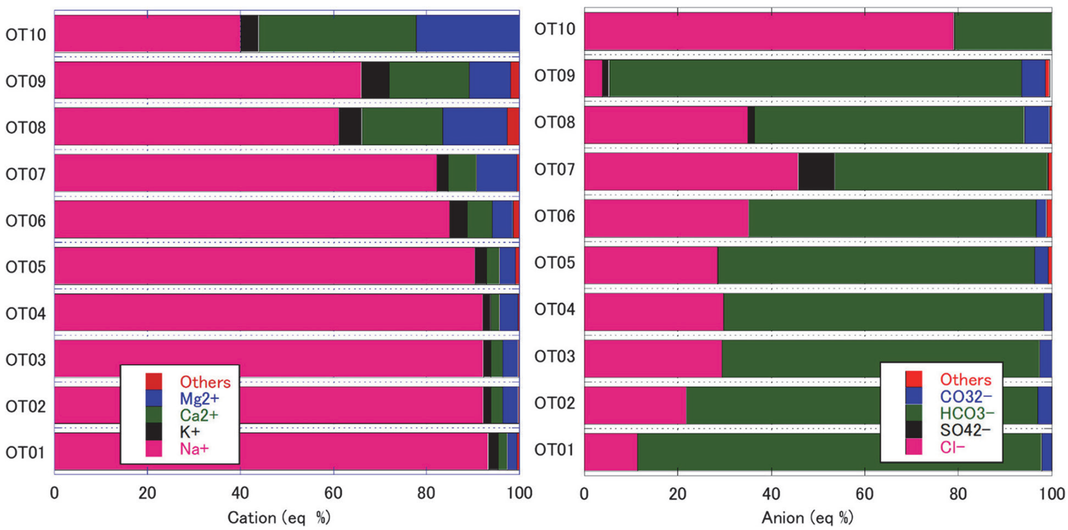

Fig. 5　Major ionic components in Kuroyu hot springs from the Ohta-ku area in Tokyo

explained as follows.

Usually, origin of water in alkaline hot springs with low dissolved matter is ascribed to meteoric water based on δD and δ^{18}O isotope ratios (Seki *et al.*, 2004). For pH increase, water reacts with granitic rocks (Takamatsu *et al.*, 1981) and felsic rocks (Ichikuni *et al.*, 1982) under limited carbon dioxide supply. In addition, Iwatsuki and Yoshida (1999) reported alteration of plagioclase in deeper groundwater system. They contribute, probably, to the formation of alkaline pH of Kuroyu hot springs in the Ohta-ku area (Table 1).

Takamatsu *et al.* (1981) conducted dissolution experiments of granitic rocks in distilled water, and showed that K/Na, Ca/Na and Mg/Na weight ratios quickly decreased with decrease of distilled water/granite powder weight ratios (g/g) in 260 hours experiment (Fig. 6). This result suggests that low contribution of granitic rocks provides a mixture of Na^+, K^+, Ca^{2+} and Mg^{2+} cation Kuroyu hot springs such as OT08-OT10 samples, while high contribution of granitic rocks forms Na^+ cation dominant Kuroyu hot springs such as OT01-OT07 samples (Fig. 5). Seki *et al.* (2004) reported on the predominance of Na^+ cation that there are two processes 1) exchange reaction of kaolinite to Ca-montmorillonite, and 2) cation exchange reaction of montmorillonite. Cl^- may be derived from sedimentary environments. $NaHCO_3$-type Kuroyu hot springs of the Tokyo Bay area are formed from $CaHCO_3$-type hot springs by ion exchange reaction of Na-montmorillonite (Muramatsu, 2011). Sodium-hydrogen carbonate and sodium-hydrogen carbonate/chloride type Kuroyu hot springs may, therefore, be predominant in the Ohta-ku area. Further study on the formation mechanisms of Kuroyu hot spring quality will be required.

3.3 Transparency and humic acid contents

Very little is known on transparency of Kuroyu hot springs. Transparency of Kuroyu hot springs varied widely from 1.8 cm to >30 cm (Table 1). OT01-OT05 samples have very low transparency (1.8–7.5 cm) which is similar to the previous study of Kuroyu hot springs from

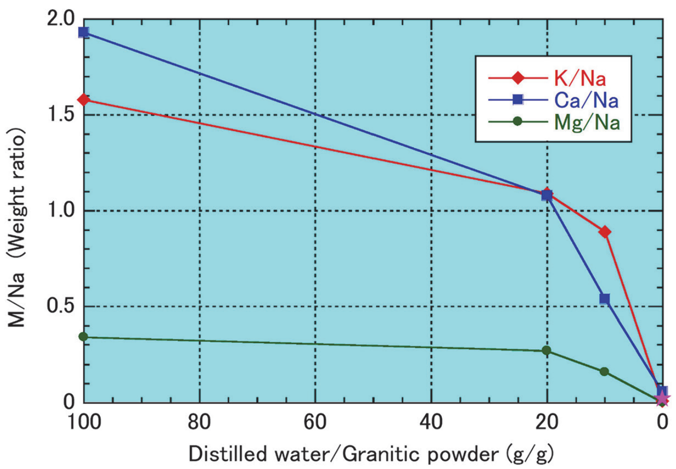

Fig. 6 Dissolution experiments of granite powder (<250 mesh) in distilled water and an alkaline hot spring (★) from Omo River area in Yamanashi Prefecture (plotted from data of Takamatsu *et al.*, 1981).

Kamata area ranging from 3.0 to 9.3 cm (Matsumoto and Ohmiya, 2019). Humic acid contents showed wide range of variation ranging from 5.8 to 325 mg/L (Table 1) which is comparable to those of Kamata hot spring samples ranging from 85 to 217 mg/L (Matsumoto and Ohmiya, 2019). Kanroji (1999a, 1999b, 2010) reported humic acid contents in Tokyo since 1950th and obtained less than 1 mg/L–490 mg/L. Humic acid contents of Mohl hot springs in Hokkaido and Kuroyu hot springs in Tokyo Bay area were 0.2–70 mg/kg (Aoyanagi *et al*., 2005 ; Takano *et al*., 2016) and 0.34–194 mg/L (Imahashi, 2015), respectively.

The Log-transparency and humic acid contents in Kuroyu hot springs in the Ohta-ku area are inversely correlated with high correlation coefficient of $r^2 = 0.965$ (Fig. 7). This result revealed that humic acid contents reflect color level of Kuroyu hot springs.

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. This is the first report of fatty acids in organic solvent extracts in 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 OT01 sample, together with branched (*iso*- and *anteiso*-C_{13-17}), and monounsaturated fatty acids ($C_{16:1}$ and $C_{18:1}$, carbon chain length : number of unsaturation, Fig. 8). Four $C_{16:1}$ peaks and two $C_{18:1}$ peaks were found in the gas chromatogram, although no polyunsaturated fatty acids were detected in OT01 sample (Fig. 9). To determine double bond position

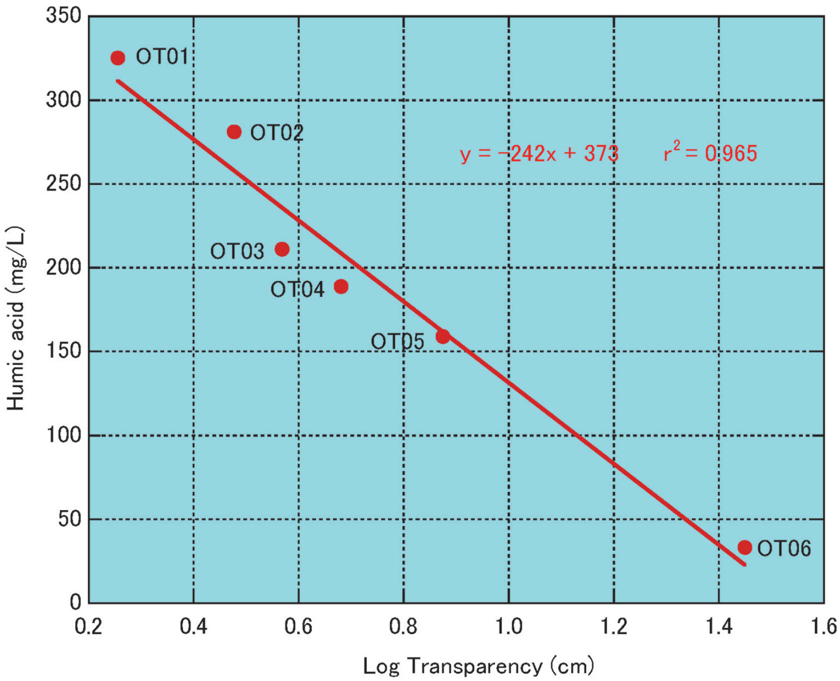

Fig. 7 Correlation between Log-transparency and humic acid contents in Kuroyu hot springs from the Ohta-ku area in Tokyo

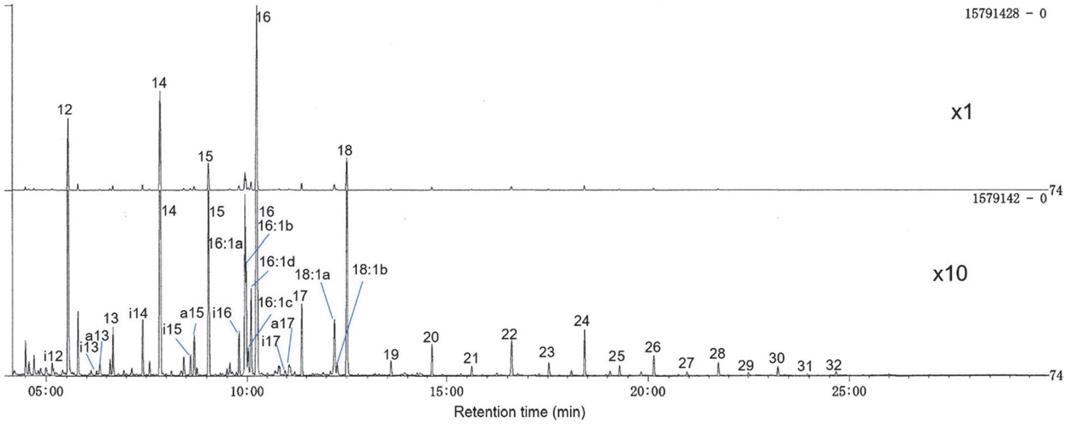

Fig. 8　Mass chromatogram of fatty acids found in ethyl acetate extract from OT01 sample in the Ohta-ku area, Tokyo. Arabic figures on the peaks denote carbon chain length of *n*-alkanoic acids. i and a are *iso*- and *anteiso*-branched acids, respectively. 16:1a–16:1d and 18:1a–18:1b are carbon chain length : number of unsaturation.

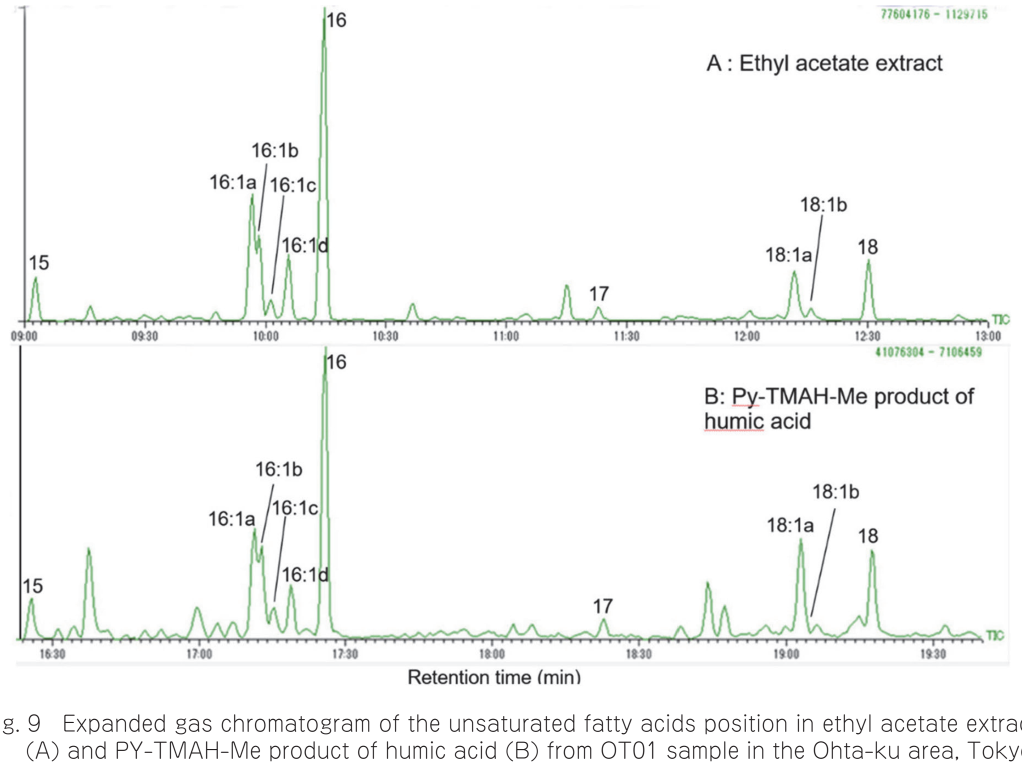

Fig. 9　Expanded gas chromatogram of the unsaturated fatty acids position in ethyl acetate extract (A) and PY-TMAH-Me product of humic acid (B) from OT01 sample in the Ohta-ku area, Tokyo.

of these peaks, we calculated equivalent carbon chain length (ECL) of the methyl esters of fatty acids (Table 2, Christe, 1989). The peaks $C_{16:1a}$, $C_{16:1b}$, $C_{16:1c}$, $C_{16:1d}$, $C_{18:1a}$ and $C_{18:1b}$ coincided with and tentatively identified to *n*-$C_{16:1}$ (9, double bond position), *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), respectively (Table 2). Normal-$C_{16:1}$(7), *n*-$C_{16:1}$(9), *n*-$C_{18:1}$(9) and *n*-$C_{18:1}$(11)

Table 2 Equivalent carbon chain length (ECL)* of *n*-alkenoic acids found in ethyl acetate extracts and Py-TMAH-Me products of humic acid.

Peak	*n*-Alkenoic acid	ECL*	Ethyl acetate extract	Py-TMAH-Me product of humic acid
16:1a	16:1(9)	15.76	15.75	15.76
16:1b	16:1(8?)	No datum	15.78	15.79
16:1c	16:1(7)	15.83	15.82	15.83
16:1d	16:1(5)	15.92	15.89	15.89
18:1a	18:1(11)	17.72	17.73	17.73
18.1	18.1(9)	17.73	Not detected	Not detected
18:1b	18:1(7)	17.78	17.79	17.79

*Christe (1989).

have been detected in Lake Yugama of Kusatsu-shirane Volcano, but no polyunsaturated fatty acids are found in the lake (Matsumoto and Watanuki, 1992).

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 3). 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 47.97–77.51%, 2.69–5.49%, 2.69–3.68% and 15.89–46.32%, respectively (Table 3). Short-chain *n*-alkanoic acids were most dominant fatty acids in all Kuroyu hot springs. Unexpectedly, *n*-alkenoic acids are abundant in OT04, OT02 and OT01 samples. These fatty acid compositions are similar to those in Toyotomi hot springs (Matsumoto *et al.* (2019), and inland waters from Ogasawara Islands (Matsumoto, 1981) and hydrothermal sediments of Tateyama (Matsumoto and Watanuki, 1990). Total fatty acid concentration ranged from 17.0 to 77.1 μg/L (Table 3). They are similar to those in some unpolluted inland waters from Ogasawara Islands (Matsumoto, 1981).

This is the first report of sterols of organic solvent extracts in Kuroyu hot springs. Cholesta-5, 22-dien-3*β*-ol, cholesterol, cholestanol, brassicasterol, 24-methyl-cholesterol, 24-methylcholestanol, 24-ethylcholesterol and 24-ethylcholestanol were found in OT01 sample from the Ohta-ku area (Fig. 10). The most abundant sterol was cholesterol in all Kuroyu hot springs (40.9–77.5%) and thus C_{27} sterol was most abundant in all Kuroyu hot springs (53.1–85.6%, Table 4). Total sterol concentrations ranged from 1.74 to 8.41 μg/L (Table 4). These concentrations are very low as compared with polluted Tamagawa River waters (20–29 μg/L, Ogura *et al.*, 1975).

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 (*iso*- and *anteiso*-C_{13-17}), and alkenoic acids (four peaks $C_{16:1}$ and two peaks $C_{18:1}$), although no polyunsaturated fatty acids were detected in OT01 sample as in the case of ethyl acetate extract, Fig. 11). Interestingly, the pattern of alkenoic acids in Py-TMAH-Me product of humic acid is similar to that of ethyl acetate extract (OT01, Fig. 8). Four $C_{16:1}$ peaks and two $C_{18:1}$ peaks in the gas chromatogram (Fig. 9) were tentatively identified as in the case of ethyl acetate extract, as

Table 3　Fatty acid composition of ethyl acetate extracts in Kuroyu hot springs from the Ohta-ku area in Tokyo.

Sample No.	OT01	OT02	OT03	OT04	OT05
n-Short (%)					
12	9.97	5.27	5.51	4.64	11.95
13	0.66	0.36	0.70	0.48	0.78
14	14.28	10.79	14.59	7.19	16.25
15	3.69	3.45	4.01	1.80	3.23
16	25.45	29.96	30.20	24.98	37.04
17	0.99	0.96	1.26	0.72	1.02
18	4.60	9.59	7.25	7.99	7.05
19	0.22	0.17	0.26	0.18	0.21
Total *n*-short(%)	59.87	60.55	63.77	47.97	77.51
n-Long (%)					
20	0.51	0.54	0.68	0.52	0.58
21	0.16	0.09	0.18	0.15	0.15
22	0.67	0.45	0.80	0.43	0.45
23	0.25	0.15	0.30	0.18	0.17
24	1.02	0.15	1.20	0.61	0.70
25	0.28	0.17	0.32	0.13	0.16
26	0.57	0.38	0.68	0.27	0.26
27	0.12	0.05	0.13	0.08	0.06
28	0.44	0.24	0.42	0.15	0.23
29	0.12	0.07	0.11	0.05	0.06
30	0.39	0.19	0.33	0.15	0.18
31	0.07	0.03	0.08	0.00	0.04
32	0.19	0.17	0.17	0.09	0.17
33	0.03	0.00	0.00	0.00	0.00
34	0.11	0.00	0.11	0.09	0.00
Total *n*-long (%)	4.93	2.69	5.49	2.89	3.22
Branched (%)					
i13	0.17	0.36	0.22	0.21	0.42
i14	0.05	0.11	0.06	0.08	0.09
i15	0.78	0.70	0.72	0.44	0.75
i16	0.23	0.60	0.72	0.68	0.33
i17	0.64	0.82	0.73	0.39	0.72
a13	0.08	0.12	0.10	0.10	0.12
a15	0.16	0.16	0.15	0.16	0.24
a17	0.57	0.82	0.70	0.76	0.72
Total branched (%)	2.69	3.68	3.40	2.82	3.38
n-Alkenoic (%)*					
16:1(9)	11.66	9.88	12.42	11.23	4.54
16:1(8?)	7.76	3.88	3.38	20.58	1.19
16:1(7)	1.68	1.10	1.69	6.07	0.36
16:1(5)	5.99	1.68	2.42	2.36	0.30
18:1(9)	4.35	15.15	6.33	4.68	8.36
18:1(7)	1.07	1.39	1.11	1.40	1.13
Total *n*-Alkenoic (%)	32.51	33.08	27.35	46.32	15.89
Total(%)	100.00	100.00	100.00	100.00	100.00
Concentration (μg/L)	77.10	23.30	33.50	34.10	17.00

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

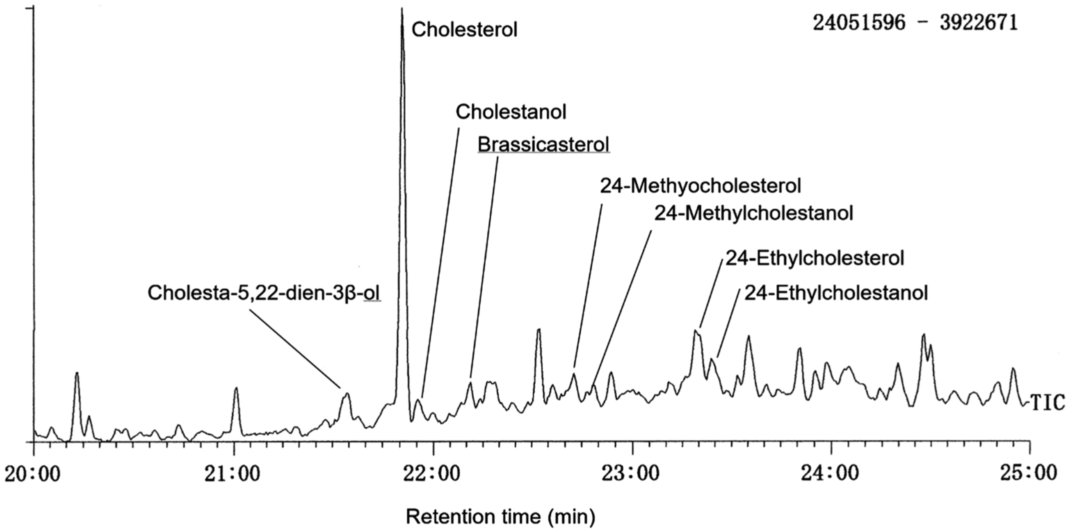

Fig. 10　Gas chromatogram of sterol TMS derivatives of ethyl acetate extract from OT01 sample in the Ohta-ku area, Tokyo.

Table 4　Sterol composition of ethyl acetate extracts in Kuroyu hot springs from the Ohta-ku area in Tokyo

Sample No.	OT01	OT02	OT03	OT04	OT05
Sterol (%)					
Stenol					
Cholesta-5,22-dien-3β-ol	5.0	3.9	4.0	6.9	6.1
Cholesterol	58.9	77.5	65.5	40.9	51.7
Brassicasterol	5.7	2.5	3.5	6.9	2.8
24-Methylcholesterol	10.9	3.9	9.6	10.8	7.0
Stigmasterol	0.0	0.0	0.0	0.0	0.0
24-Ethylcholesterol	8.7	5.3	8.1	18.2	23.7
Stanol					
Cholestanol	3.1	2.7	3.0	4.8	2.4
24-Methylcholestanol	3.2	1.8	2.0	5.3	3.7
24-Ethylcholestanol	4.6	2.4	4.2	6.2	2.6
Total (%)	100.0	100.0	100.0	100.0	100.0
C_{27}–C_{29} sterol (%)					
C_{27} sterol	69.4	85.6	74.2	53.1	59.4
C_{28} sterol	15.8	6.1	12.5	18.7	11.7
C_{29} sterol	14.8	8.3	13.3	28.3	28.9
Concentration (μg/L)	8.41	2.52	3.91	1.74	2.02

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) (Table 2).

The major fatty acids (>10%) were *n*-C_{14}, *n*-C_{16}, *n*-$C_{16:1}$(9), *n*-$C_{16:1}$(8?) and/or *n*-$C_{16:1}$(5) (Table 5). The most predominant fatty acid was *n*-C_{16}, *n*-$C_{16:1}$(9) or *n*-$C_{16:1}$(8?). As compared with fatty acids

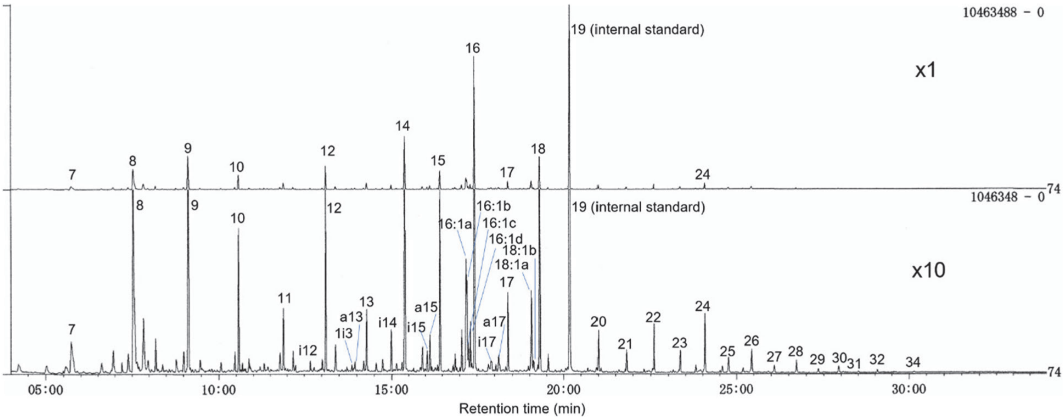

Fig. 11　Mass chromatogram of fatty acids (m/z 74) obtained by Py-TMAH-Me-GC/MS of humic acid from OT01 sample. Arabic figures on the peaks are same as in Fig. 7.

of ethyl acetate extracts, abundance of *n*-$C_{16:1}$(9) or *n*-$C_{16:1}$(8?) is originated from degradation products of humic acid. Fatty acid compositions revealed that short-chain *n*-alknoic acids (*n*-C_{12}–*n*-C_{34}), long-chain *n*-alkanoic acids (*n*-C_{20}–*n*-C_{34}), branched acids (*n*-C_{13}–*n*-C_{17}) and *n*-alkenoic acids (*n*-$C_{16:1}$, *n*-$C_{18:1}$) were 30.78–47.31, 2.05–16.19, 1.34–4.86 and 17.03–64.05%, respectively (Table 5). Very short-chain *n*-alkanoic acids (*n*-C_7–*n*-C_{11}) were present in all Kuroyu hot springs. Short-chain *n*-alkanoic acids were most dominant in OT01, OT03, OT05, OT06 and OT07 samples, while interestingly *n*-alkenoic acids were most abundant in OT02, OT08, OT09 and OT10 samples (Table 5). 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.*, 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 5). Humic acid is, therefore, mainly derived from algae. Unusual abundance of *n*-monousaturated 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., Weete, 1976 ; Kawamura *et al.*, 1980 ; Kawamura and Ishiwatari, 1981) are

Table 5　Fatty acid composition of Py-TMAH-Me-GC/MS products of Kuroyu hot springs from the Ohta-ku area in Tokyo.

Sample No.	OT01	OT02	OT03	OT04	OT05	OT06	OT07	OT08	OT09	OT10
n-Short (%)										
7	0.59	0.58	0.65	0.18	0.88	0.61	1.08	0.10	0.40	0.15
8	3.72	2.79	2.75	0.65	3.50	3.19	4.07	0.28	1.75	0.73
9	6.01	3.46	3.78	2.45	5.94	2.03	6.73	0.75	2.62	1.15
10	2.55	1.43	2.06	1.44	3.13	2.03	3.64	0.47	1.17	0.53
11	1.14	0.75	1.07	0.65	1.54	0.46	1.91	0.19	0.38	0.13
12	1.12	2.54	3.78	4.28	4.69	3.19	4.38	1.45	2.05	3.29
13	1.11	0.79	1.03	0.80	1.25	0.59	1.80	0.25	0.44	0.34
14	9.58	11.09	14.79	8.89	8.44	3.19	8.95	9.24	6.41	13.13
15	3.41	2.31	2.41	3.26	3.28	12.23	3.46	1.33	2.01	2.08
16	23.95	14.32	19.95	18.76	19.39	17.97	19.13	15.78	22.59	13.55
17	1.44	0.90	1.03	1.03	1.39	0.93	1.82	0.44	0.82	0.45
18	5.85	3.70	4.30	4.08	6.22	4.98	4.94	2.29	4.44	2.50
Total *n*-short(%)	60.47	44.67	57.63	46.48	59.65	51.38	61.91	32.57	45.10	38.02
Total 12-18 (%)	46.45	35.65	47.31	41.11	44.66	43.07	44.47	30.78	38.77	35.34
n-Long (%)										
20	0.89	0.88	0.82	0.73	1.34	0.91	1.70	0.33	0.66	0.33
21	0.54	0.42	0.52	0.36	0.77	0.33	1.11	0.14	0.20	0.08
22	1.19	1.63	1.28	0.78	2.47	1.73	2.25	0.41	0.66	0.37
23	0.59	0.49	0.55	0.36	0.82	0.44	1.21	0.15	0.28	0.14
24	1.86	1.40	1.76	1.07	2.54	1.96	2.75	0.40	1.15	0.73
25	0.47	0.37	0.49	0.29	0.66	0.36	0.87	0.10	0.30	0.15
26	0.81	0.66	0.85	0.50	1.09	0.91	1.41	0.17	0.59	0.32
27	0.23	0.28	0.30	0.11	0.45	0.25	0.68	0.07	0.18	0.02
28	0.55	0.56	0.67	0.25	1.08	0.77	1.29	0.12	0.41	0.08
29	0.14	0.25	0.23	0.02	0.37	0.25	0.55	0.03	0.16	0.03
30	0.34	0.53	0.48	0.06	0.86	0.80	1.07	0.08	0.35	0.06
31	0.04	0.15	0.12	0.01	0.24	0.21	0.34	0.02	0.02	0.01
32	0.12	0.30	0.26	0.02	0.58	0.54	0.57	0.01	0.14	0.04
33	0.00	0.05	0.06	0.00	0.06	0.12	0.12	0.01	0.01	0.00
34	0.00	0.08	0.12	0.00	0.16	0.27	0.27	0.00	0.10	0.00
Total *n*-long (%)	7.78	8.04	8.49	4.55	13.51	9.86	16.19	2.05	5.20	2.36
Branched (%)										
i12	0.16	0.14	0.14	0.16	0.22	0.12	0.43	0.04	0.11	0.05
i13	0.13	0.12	0.11	0.12	0.19	0.13	0.25	0.03	0.22	0.02
i14	1.01	0.61	0.38	0.57	0.88	0.57	0.96	0.27	0.58	0.36
i15	0.35	1.15	0.52	0.45	0.91	1.23	0.65	0.25	0.38	0.24
i16	0.43	0.37	0.50	0.55	0.66	0.59	0.93	0.28	0.41	0.32
i17	0.16	0.18	0.12	0.16	0.28	0.21	0.23	0.08	0.11	0.06
a13	0.16	0.13	0.14	0.16	0.20	0.09	0.25	0.03	0.11	0.05
a15	0.64	0.60	0.48	0.65	0.81	0.52	0.83	0.25	0.44	0.32
a17	0.28	0.14	0.15	0.16	0.31	0.14	0.34	0.11	0.16	0.15
Total branched (%)	3.31	3.45	2.54	3.00	4.46	3.61	4.86	1.34	2.52	1.58
n-Alkenoic (%)*										
16:1(9)	8.51	12.70	11.25	12.07	7.50	12.43	4.35	20.82	3.10	17.05
16:1(8?)	7.45	14.39	9.46	18.97	4.85	8.58	4.88	20.46	30.09	17.19
16:1(7)	2.34	4.23	3.06	5.22	2.28	2.96	1.30	6.46	3.10	9.54
16:1(5)	3.51	7.09	4.37	3.47	2.72	4.90	1.08	10.97	1.86	11.84
18:1(11)	6.55	2.79	2.72	5.42	4.63	4.17	4.35	3.13	3.64	1.17
18:1(7)	0.08	2.63	0.48	0.82	0.41	2.12	1.08	2.21	5.39	1.25
Total *n*-alkenoic (%)	28.44	43.84	31.34	45.97	22.39	35.15	17.03	64.05	47.18	58.04
Total(%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

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

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^{2}d. 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^{2}d, 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 of polyunsaturated acids. 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.

Sterols (stenols and stanols) are widely distributed in lacustrine and marine environments. Chlolesterol 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} sterols (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). This is derived from algae including plankton with some contribution of diatom and vascular plants.

Stanols are found in certain microalgae such as 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 Ohta-ku area of Tokyo 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. These hot springs may be formed by contribution levels of granitic rocks and ion exchange reaction of montmorillonite.

(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 algae with some contribution of vascular plants and bacteria. Unusual abundance of *n*-mono-unsaturated fatty acids can be explained by the degradation loss of polyunsaturated fatty acids in the sedimentary environments.

(7)　Well depths (30–120 m) imply that sedimentary environments are shallow Paleo Tokyo Bay of Shimousa Group and marine land shelf slope of Kazusa Group in ages 0.5–1 Ma.

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