



Application of Column Chromatography for Accurate Determination of the Carbon Isotopic Compositions of *n*-alkanes in Diverse Environmental Samples

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Abstract

The carbon isotopic compositions ($\delta^{13}\text{C}$) of *n*-alkanes in various environmental samples have been previously proposed as suitable fingerprints for assessing the origin of organic matter (OM) in diverse environmental systems. However, with respect to using gas chromatography–combustion–isotope ratio mass spectrometry for the carbon isotopic analysis of *n*-alkanes, analytical uncertainty may often be caused by the co-elution of interfering unsaturated compounds (e.g., aromatic and branched compounds). Hence, we propose a simple but reliable method for purification that uses column chromatography. The performance of two different solid stationary phases (i.e., aluminum oxide and Ag^+ -impregnated silica) was compared in terms of their capacity to eliminate unsaturated compounds from total hydrocarbons and thus increase the precision of $\delta^{13}\text{C}$ measurements. Compared to the use of an activated aluminum oxide column, elution from an Ag^+ -impregnated silica column allows more effective isolation of individual *n*-alkanes, which results in more precise $\delta^{13}\text{C}$ measurements for diverse environmental samples. Thus, Ag^+ -impregnated silica column separation can be effective as a routine experimental technique for increasing the accuracy of the $\delta^{13}\text{C}$ values for *n*-alkanes in OM, which includes a large proportion of unsaturated compounds.

Keywords Ag^+ -impregnated silica column · Carbon isotopic composition ($\delta^{13}\text{C}$) · Gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS) · *N*-alkanes · Organic matter

1 Introduction

Acyclic saturated hydrocarbons (i.e., *n*-alkanes) consist of straight carbon chains with even and odd numbers of carbon atoms. The various chain lengths of *n*-alkanes are ubiquitously present in natural environments (e.g., Gelpi et al. 1970; Canuel and Martens 1993; Ficken et al. 2000). For instance, short-chain *n*-alkanes ($< \text{C}_{20}$) are mainly derived from bacteria and algae (e.g., Cranwell et al. 1987; Pisani et al. 2013). The *n*-alkanes with medium-chain lengths (i.e., C_{20} – C_{25}) are dominant components of submerged and

floating aquatic macrophytes (e.g., Cranwell et al. 1987; Ficken et al. 2000). Long-chain *n*-alkanes (C_{25} – C_{35}) with strong odd-to-even carbon preference mainly originate from terrestrial sources, such as epicuticular leaf waxes (e.g., Bray and Evans 1961; Eglinton and Eglinton 2008). Therefore, discriminative *n*-alkane patterns derived from various organic matter (OM) sources are frequently used to assess the origin of OM in natural environments (e.g., Bourbonnière and Meyers 1996; Meyers 2003; Eglinton and Eglinton 2008; Derrien et al. 2017). However, *n*-alkanes and their precursors can be produced from a wide variety of OM sources; therefore, *n*-alkane patterns for apportioning OM can result in inaccurate estimations of the relative contributions of various sources, such as algae, bacteria, and terrestrial vascular plants.

To obtain more accurate information on the sources and contributions of OM, the $\delta^{13}\text{C}$ values of individual *n*-alkanes have often been determined for various materials in natural environments (Lichtfouse et al. 1994; Chikaraishi and Naraoka 2007; Cooper et al. 2015). Because *n*-alkanes are less reactive than bulk OM and other lipid classes (Meyers

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and Ishiwatari 1993; Meyers 2003), their isotopic compositions have been proposed as suitable fingerprints for the apportionment of sources in complex environments (McCornachie and Peticrew 2006; Gibbs 2008; Cooper et al. 2015; Lee et al. 2019). However, one potential problem is that, during gas chromatographic separation, *n*-alkanes are often co-eluted with unsaturated (i.e., aromatic and branched) hydrocarbons that can form humps in the chromatogram (e.g., Mille et al. 2007; Fiselier et al. 2009; Moret et al. 2011). In this case, conventional organic geochemical methods must be modified to distinguish between the unsaturated and saturated components. Research toward this goal has improved isolation techniques through the use of on-line (e.g., high-performance liquid chromatography–gas chromatography (HPLC–GC) or high-performance liquid chromatography–high-performance liquid chromatography–gas chromatography (HPLC–HPLC–GC)) and off-line approaches (e.g., Biedermann et al. 2009; Moret et al. 2011). Although on-line techniques are highly reproducible and capable of processing a large number of samples per day, the necessary instrumentation is only available in a few laboratories. Thus, a more broadly applicable process involving a simple experimental approach is necessary for the effective isolation of *n*-alkanes in diverse environmental samples.

In this study, our main objective was to effectively isolate saturated fractions from the total hydrocarbons preserved in various environmental samples (e.g., marine/lake sediment, soil, terrestrial C₃ plants, and suspended particulate matter). We then evaluated the ability of the proposed approach to more precisely measure the $\delta^{13}\text{C}$ of *n*-alkanes by applying it using two different solid columns. Our approach will facilitate determination of the source apportionment of OM in complex environmental settings.

2 Materials and Methods

2.1 Sample Collection

Two marine sediment samples were collected from the East Sea of Korea (ES14-BC01; 37°12.05'N, 130°25.0'E; 2160 m water depth) in May 2014, and the Chukchi Sea (ARA01B/04MUC-01; 73°44.10'N, 167°0.2'E; 43 m water depth) in August 2010 with R/V Araon using a box core and a multi-core, respectively. One soil sample was collected from a wooded area on the Hanyang University campus in May 2015. One lake sediment sample was obtained from Lake Soyang (30 m water depth) in May 2014 using a grab. One terrestrial C₃ plant (viz. *Pinus densiflora*) sample was collected at Hanyang University in October 2016, and thoroughly washed with distilled water to remove contaminants. One suspended particulate matter (SPM) sample was collected from the Geum River in August 2016 by filtering the

water through a 0.45 μm GF-5 filter (diameter: 125 mm, Whatman). All samples were stored at $-20\text{ }^\circ\text{C}$ prior to lipid analysis.

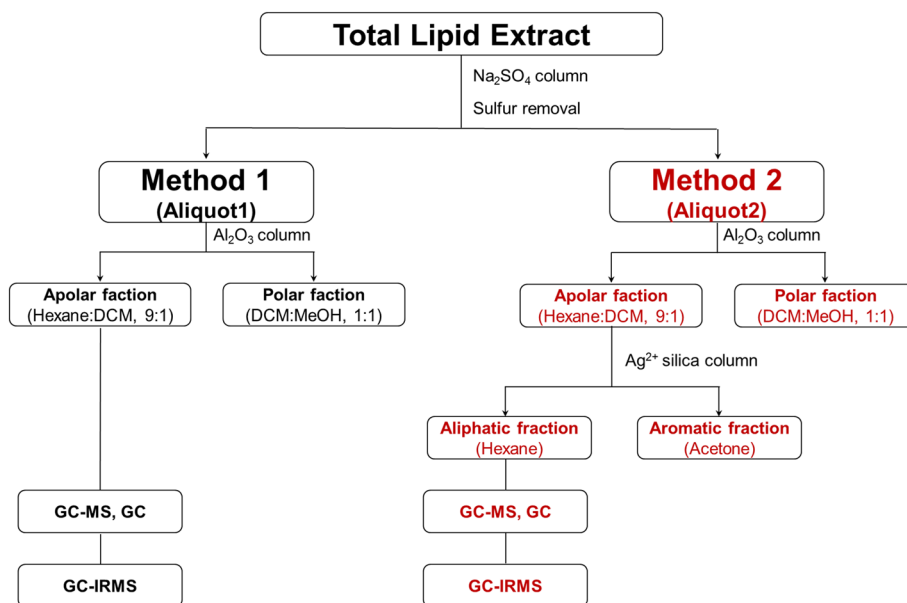
2.2 Lipid Extraction and Purification

All chemicals used for extraction and purification in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). For column chromatography, aluminum oxides (particle size: 0.063–0.200 mm) were purchased from Merck (Darmstadt, Germany), and Ag⁺-impregnated silica gels (particle size: 50 μm) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All environmental samples were freeze-dried, and sediment and plant samples were further ground using a mortar and pestle. Homogenized (2–10 g) and SPM samples were extracted by applying an accelerated solvent extractor (ASE 200, Dionex, California, USA) with a solvent mixture of dichloromethane (DCM) and methanol (MeOH) (9:1, v:v) at 100 $^\circ\text{C}$, and a pressure of 7.6×10^6 Pa. The total lipid extract (TLE) was dried by passing it through a sodium sulfate (Na₂SO₄) column; it was then treated with a tetrabutylammonium sulfite reagent to remove elemental sulfur. An aliquot of TLE was chromatographically separated into apolar and polar fractions on an alumina column (2 h activation at 150 $^\circ\text{C}$) by applying solvents with increasing polarity. The apolar fraction containing *n*-alkanes was eluted using hexane:DCM (9:1, v:v), and the polar fraction was obtained by applying DCM:MeOH (1:1, v:v) as the eluent (Fig. 1). A second aliquot of TLE was chromatographically separated by applying the same procedure as mentioned above. However, the apolar fraction was further purified using an Ag⁺-impregnated silica column, with hexane and acetone as eluents, to isolate the saturated and unsaturated hydrocarbons, respectively (Fig. 1).

2.3 Gas Chromatography–Mass Spectrometry (GC–MS)

For compound identification, the apolar fractions that were separated using different solid phases were analyzed by employing a gas chromatograph (GC) connected to a mass spectrometer (MS) (QP2010 plus, Shimadzu Corporation, Kyoto, Japan) that was equipped with a fused silica capillary column (CP-sil 5 CB, 25 m length, 0.32 mm i.d., and 0.12 μm film thickness; Agilent J & W, Santa Clara, USA) with flowing helium carrier gas (1.2 mL/min). Mass scans were performed in the range of m/z 50–800, and the MS was operated at 70 eV (cycle time: 0.9 s, resolution: 1000). The samples were injected at 70 $^\circ\text{C}$ in splitless mode. The GC oven temperature was subsequently raised to 130 $^\circ\text{C}$ at a rate of 20 $^\circ\text{C}/\text{min}$, and then to 320 $^\circ\text{C}$ at 4 $^\circ\text{C}/\text{min}$, with a final hold time of 15 min. The chromatograms and mass spectra were obtained in post-run analysis using Shimadzu

Fig. 1 Schematics of experimental processes applied to isolate saturated *n*-alkanes using activated aluminum oxide (Method 1, left) and Ag⁺-impregnated silica gel (Method 2, right)



software (Shimadzu Corporation, Kyoto, Japan). Peaks were identified by comparing the experimental mass spectral fragmentation patterns to those existing in the Shimadzu software library, and by comparing the retention time to those corresponding to the standard *n*-alkane mixture.

2.4 Gas chromatography–Combustion–Isotope Ratio Mass Spectrometry (GC–C–IRMS)

The $\delta^{13}\text{C}$ values for individual *n*-alkanes were determined using gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS), as described by Lee et al. (2018). An IRMS instrument (Isoprime, GV Instruments, Manchester, UK) was connected to a gas chromatograph (Hewlett Packard 6890N series, Agilent Technologies, Santa Clara, USA) by a combustion interface (i.e., a glass tube packed with copper oxide, operated at 850 °C). The samples were passed through the same capillary column as that described for GC–MS analysis, and the same temperature conditions were applied. Reference CO₂ gas of known $\delta^{13}\text{C}$ value was pulsed at the beginning and end of each sample run. Isotopic values were expressed as $\delta^{13}\text{C}$ values per mil (‰) relative to the Vienna-Pee Dee Belemnite (VPDB), and calculated as follows:

The $\delta^{13}\text{C}$ values were further corrected by applying a certified *n*-alkane standard (Schimmelmann alkane mixture type A6, Indiana University, USA). Using the standard *n*-alkane mixture, comparison of the $\delta^{13}\text{C}$ values to the certified values yielded a correlation coefficient (r^2) higher than 0.99. To monitor the accuracy of the isotopic measurements, standards with known $\delta^{13}\text{C}$ values were repeatedly analyzed every 5–6 sample runs. Standard deviations of carbon

isotope measurements were generally higher than $\pm 0.4\text{‰}$, as determined by repeated injections of the standard.

3 Results and Discussion

First, using the certified *n*-alkane mixture standard, we compared the isotopic variations obtained from two different stationary phases (i.e., aluminum oxide and Ag⁺-impregnated silica). The $\delta^{13}\text{C}$ values ranged from $-33.7 \pm 0.1\text{‰}$ to $-27.3 \pm 0.4\text{‰}$ for Method 1, and $-33.7 \pm 0.2\text{‰}$ to $-27.3 \pm 0.2\text{‰}$ for Method 2 (Table 1). Comparison to the certified $\delta^{13}\text{C}$ values revealed no significant differences for Method 1 or 2. Thus, we argue that the use of column chromatography (either Method 1 or Method 2) has a negligible effect on the isotopic ratio of the eluted fraction.

Second, GC–MS was applied to the diverse environmental samples; the chromatograms showing patterns of *n*-alkanes that were representative of Methods 1 and 2 were then compared (Fig. 2). The peaks of individual *n*-alkanes were more distinct when Method 2 was applied; this is because the Ag⁺-impregnated silica column removed humps in the chromatograms (Fig. 2). Our results are in good agreement with those of previous studies, which demonstrated that the Ag⁺ ion has a chemical affinity for adsorbing double-bond-containing compounds (e.g., Moret et al. 2011). In fact, non-targeted compounds of the investigated samples (especially terrestrial C₃ plants and SPM) seem to have been more effectively removed after Ag⁺-impregnated silica column separation. Thus, this technique appears to be useful for secondary sample purification.

Third, we compared the $\delta^{13}\text{C}$ values for *n*-alkanes detected in the environmental samples (Table 2; Fig. 3). In

Table 1 Comparison of carbon isotopic compositions of the certified *n*-alkanes standard to the results of two different column chromatography techniques (Method 1: activated aluminum oxide; Method 2: Ag⁺-impregnated silica)

<i>n</i> -alkanes	Certified <i>n</i> -alkanes standard (<i>n</i> =5)		Method 1 (<i>n</i> =5)		Method 2 (<i>n</i> =5)	
	$\delta^{13}\text{C}$ (‰ VPDB)	SD	$\delta^{13}\text{C}$ (‰ VPDB)	SD	$\delta^{13}\text{C}$ (‰ VPDB)	SD
C16	-26.8	0.4	-27.3	0.4	-27.3	0.2
C17	-32.0	0.4	-32.0	0.1	-32.0	0.2
C18	-32.4	0.2	-32.6	0.2	-32.6	0.4
C19	-32.0	0.3	-32.0	0.2	-32.0	0.2
C20	-33.6	0.3	-33.7	0.1	-33.7	0.2
C21	-29.1	0.2	-29.3	0.1	-29.3	0.2
C22	-33.2	0.2	-33.4	0.2	-33.4	0.3
C23	-32.8	0.2	-33.0	0.2	-33.0	0.3
C24	-31.8	0.2	-31.9	0.2	-31.9	0.3
C25	-28.6	0.2	-28.8	0.1	-28.8	0.1
C26	-32.4	0.3	-32.6	0.1	-32.6	0.3
C27	-30.2	0.3	-30.4	0.2	-30.4	0.3
C28	-32.6	0.3	-32.7	0.2	-32.7	0.2
C29	-29.1	0.2	-29.2	0.2	-29.2	0.4
C30	-29.6	0.2	-29.6	0.3	-29.6	0.2

Fig. 2 GC–MS chromatograms of individual *n*-alkanes detected in various environmental samples: **a** marine sediment 1, **b** marine sediment 2, **c** soil, **d** lake sediment, **e** terrestrial C₃ plant, and **f** suspended particulate matter

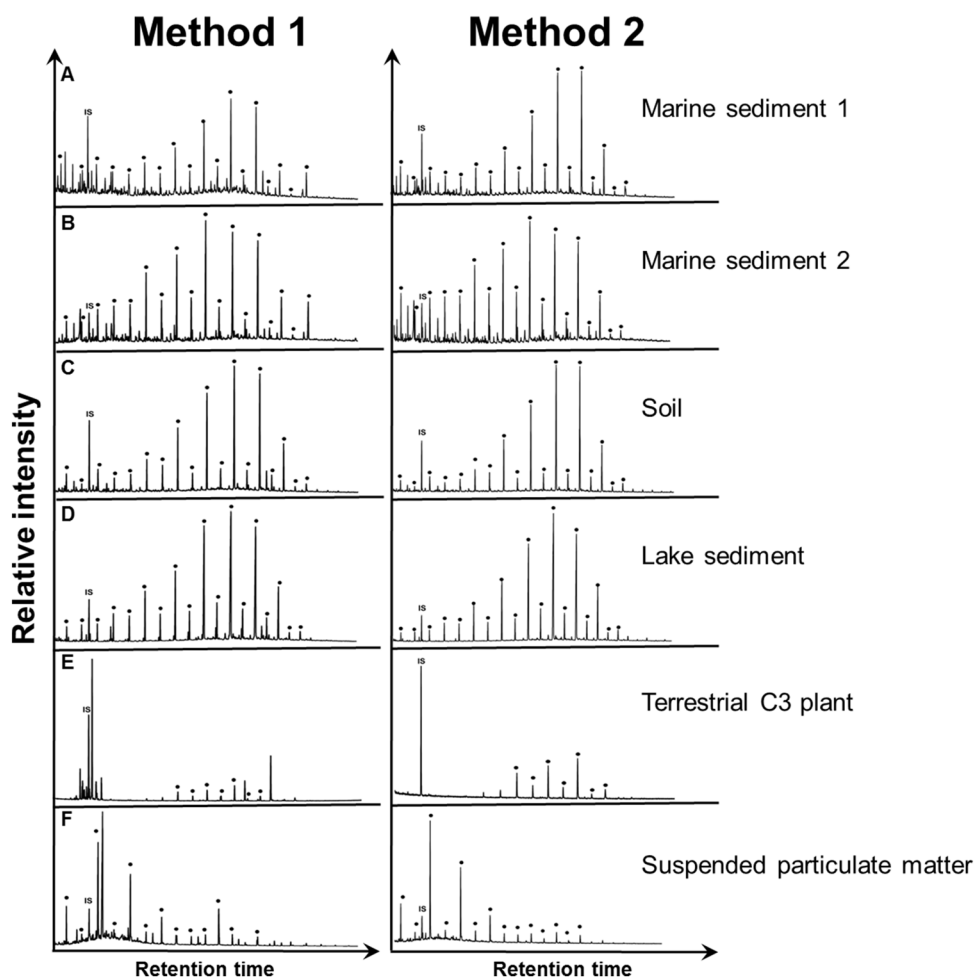


Table 2 *n*-alkane carbon isotopic compositions ($\delta^{13}\text{C}$, ‰ VPDB) of each sample obtained by the activated aluminum oxide column chromatography method (i.e., Method 1) and the Ag^+ -impregnated silica column chromatography method (i.e., Method 2)

<i>n</i> -alkanes	Marine sediment 1 (<i>n</i> =3)		Marine sediment 2 (<i>n</i> =3)		Soil (<i>n</i> =3)		Lake sediment (<i>n</i> =3)		Terrestrial C3 plant (<i>n</i> =3)		Suspended particulate matter (<i>n</i> =3)	
	$\delta^{13}\text{C}$ (‰ VPDB)	SD	$\delta^{13}\text{C}$ (‰ VPDB)	SD	$\delta^{13}\text{C}$ (‰ VPDB)	SD	$\delta^{13}\text{C}$ (‰ VPDB)	SD	$\delta^{13}\text{C}$ (‰ VPDB)	SD	$\delta^{13}\text{C}$ (‰ VPDB)	SD
<i>Method 1</i>												
C18	-31.0	7.4	-27.5	1.2	-29.3	4.2	-28.2	1.5	-	-	-25.9	0.9
C19	-25.0	3.4	-37.0	1.1	-	-	-30.9	0.4	-	-	-30.9	1.4
C20	-28.7	3.0	-29.2	2.1	-29.2	0.2	-28.8	0.8	-	-	-26.9	0.5
C21	-27.7	1.3	-30.0	0.4	-28.2	1.3	-31.8	0.9	-	-	-24.8	1.4
C22	-27.6	0.4	-29.2	0.5	-27.1	1.1	-31.1	1.0	-	-	-25.7	3.0
C23	-28.5	1.7	-32.2	0.1	-35.3	1.1	-32.4	0.4	-	-	-29.3	1.3
C24	-28.8	0.3	-31.4	0.1	-32.4	1.5	-31.8	1.0	-	-	-26.0	0.4
C25	-29.8	0.2	-31.7	0.1	-35.2	0.3	-32.4	0.4	-29.6	4.6	-31.3	1.5
C26	-28.7	1.4	-32.1	0.1	-33.8	2.6	-31.9	0.4	-	-	-	-
C27	-29.3	0.4	-32.4	0.3	-34.1	0.4	-32.9	0.3	-29.4	2.1	-31.6	0.2
C28	-27.7	0.9	-32.6	0.9	-29.9	0.4	-33.7	0.7	-	-	-	-
C29	-29.8	0.1	-33.2	0.4	-35.3	0.2	-34.7	0.2	-31.9	0.2	-33.3	1.1
C30	-31.8	1.9	-33.1	0.2	-36.4	3.8	-35.4	0.5	-	-	-	-
C31	-31.1	0.2	-33.7	0.2	-37.1	0.7	-35.7	0.1	-32.0	5.1	-32.3	0.6
C32	-	-	-	-	-35.0	1.6	-35.1	0.5	-	-	-	-
C33	-28.6	0.1	-33.2	0.1	-35.6	0.8	-35.8	0.4	-	-	-	-
<i>Method 2</i>												
C18	-24.0	2.5	-27.2	0.6	-25.3	3.9	-27.9	1.0	-	-	-24.2	0.8
C19	-27.5	1.2	-31.3	0.6	-	-	-30.1	0.4	-	-	-27.8	1.0
C20	-24.7	1.1	-27.3	0.9	-26.2	1.4	-28.8	0.6	-	-	-25.4	0.3
C21	-26.7	0.5	-30.6	0.7	-27.6	2.2	-31.8	0.7	-	-	-28.1	1.2
C22	-24.1	0.5	-28.9	0.2	-28.8	1.5	-30.7	0.3	-	-	-25.4	0.5
C23	-28.1	1.4	-31.5	0.2	-33.7	0.7	-32.3	0.4	-	-	-31.5	1.0
C24	-28.6	0.3	-30.8	0.4	-33.4	0.3	-31.9	0.4	-	-	-26.5	0.4
C25	-29.4	0.2	-32.0	0.4	-35.2	0.8	-32.8	0.5	-32.0	1.9	-27.8	0.9
C26	-26.9	3.0	-31.5	0.1	-31.4	2.5	-32.3	0.5	-	-	-	-
C27	-29.5	0.5	-32.3	0.2	-34.8	0.3	-33.2	0.4	-32.5	2.0	-30.9	0.5
C28	-28.6	1.7	-33.1	0.4	-32.6	3.3	-33.5	0.3	-	-	-	-
C29	-30.7	0.2	-33.1	0.1	-35.7	0.2	-34.6	0.1	-33.1	0.3	-32.3	0.4
C30	-31.6	1.0	-33.7	0.4	-36.1	2.2	-34.6	0.5	-	-	-	-
C31	-31.1	0.2	-33.8	0.1	-37.5	0.4	-35.5	0.2	-34.9	3.2	-31.1	0.4
C32	-	-	-	-	-35.2	0.1	-35.1	0.1	-	-	-	-
C33	-29.7	0.7	-33.1	0.4	-36.7	0.3	-35.4	0.3	-	-	-	-

“-” denotes “not determined”

general, the $\delta^{13}\text{C}$ values of high molecular weight *n*-alkanes (C_{25} – C_{33}) were similar between Method 1 and Method 2. However, larger differences between two methods were observed for low molecular weight *n*-alkanes (C_{18} – C_{20}).

The percent error for each sample showed that Method 2 ($\pm 0.1\text{‰}$ to $\pm 3.9\text{‰}$) was more precise than Method 1 ($\pm 0.1\text{‰}$ to $\pm 7.4\text{‰}$) (Fig. 4). Among the diverse samples, the $\delta^{13}\text{C}$ values for *n*-alkanes, as detected in the terrestrial

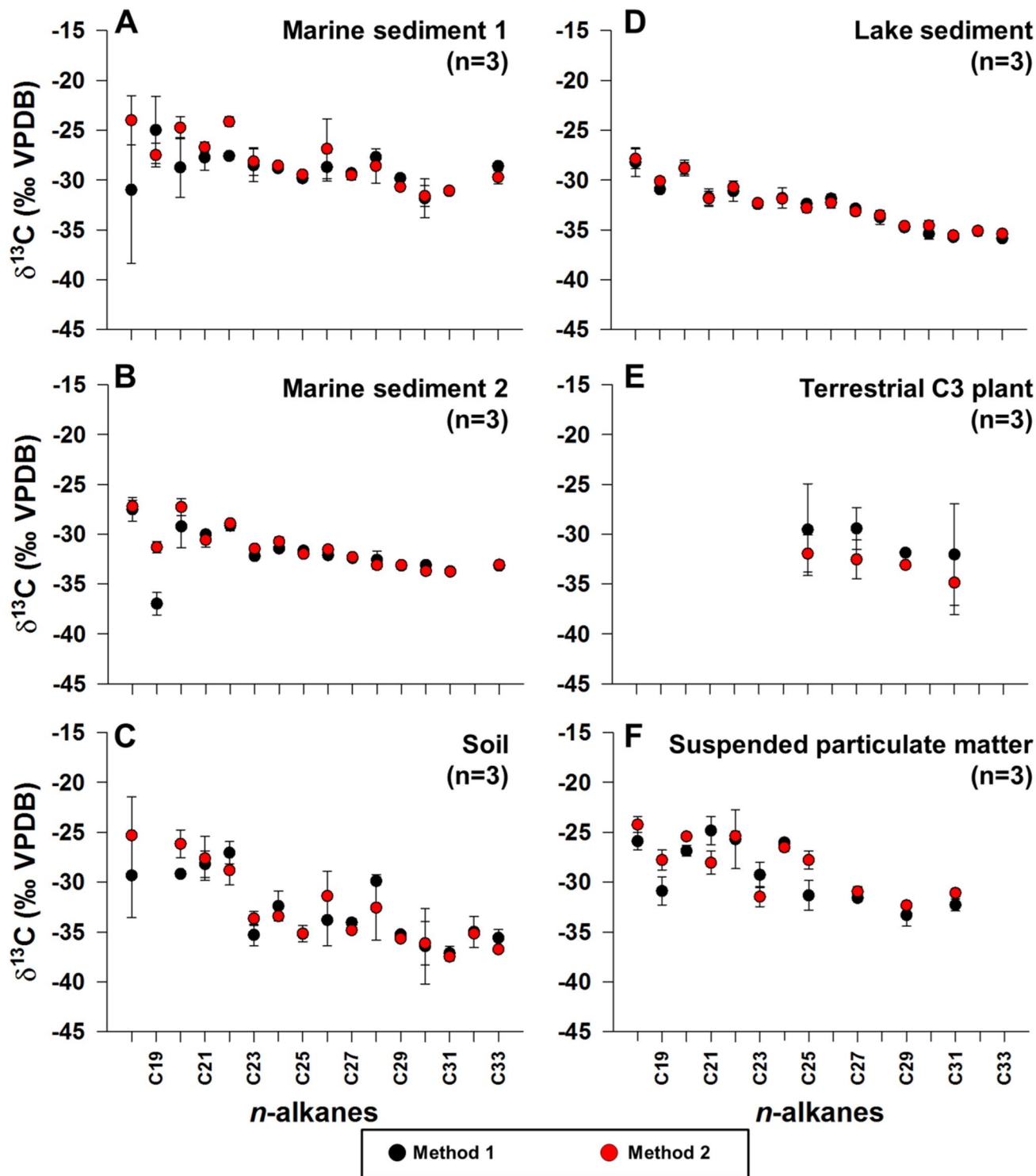


Fig. 3 $\delta^{13}\text{C}$ results for individual *n*-alkanes detected in environmental samples: **a** marine sediment 1, **b** marine sediment 2, **c** soil, **d** lake sediment, **e** terrestrial C_3 plant, and **f** suspended particulate matter. *Black and red circles* indicate $\delta^{13}\text{C}$ values for *n*-alkanes obtained by Methods 1 and 2, respectively

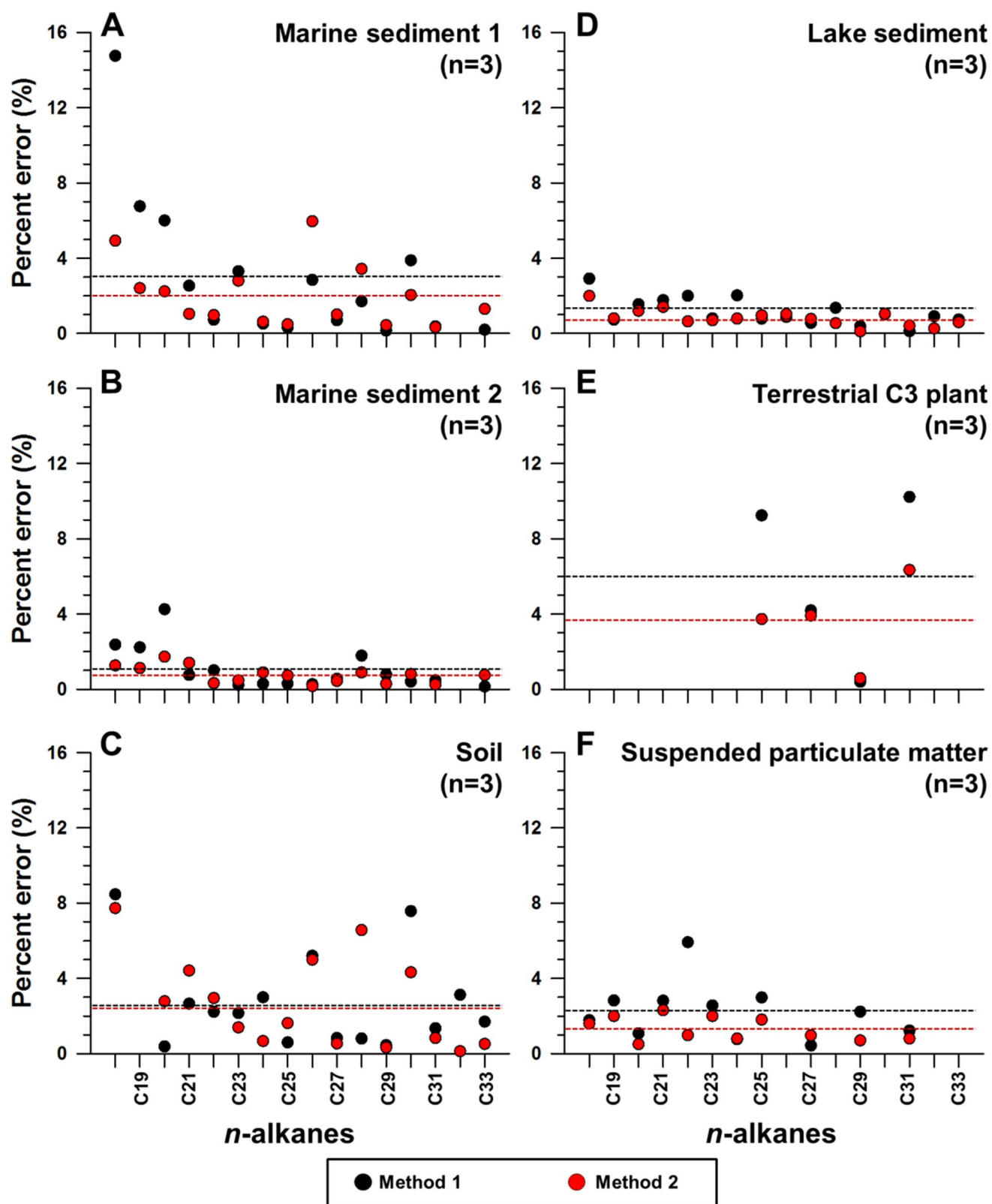


Fig. 4 Percent error of $\delta^{13}\text{C}$ measurements for individual n -alkanes calculated in environmental samples: **a** marine sediment 1, **b** marine sediment 2, **c** soil, **d** lake sediment, **e** terrestrial C₃ plant, and **f** suspended particulate matter. *Black and red dash lines* indicate the average values obtained by Methods 1 and 2, respectively

C₃ plant sample using Methods 1 and 2, were confirmed to have the largest difference ($p < 0.05$).

Previously, it has been shown that total hydrocarbons contain both *n*-alkanes and complex mixtures of unsaturated fractions (e.g., branched and cyclic compounds; Mille et al. 2007). Hence, the potential analytical uncertainty associated with the isotopic compositions of *n*-alkanes may arise from the co-elution of non-targeted unsaturated compounds in GC–C–IRMS chromatograms (Ishiwatari et al. 1994). In fact, the $\delta^{13}\text{C}$ values for the *n*-alkanes detected in the environmental samples (e.g., terrestrial C₃ plants) that were obtained by Method 1 exhibited more analytical uncertainty; this is because of the large amounts of unsaturated fractions co-eluting near C₁₈–C₂₀ (Fig. 4). Thus, the Ag⁺-impregnated silica column separation method (i.e., Method 2) can facilitate the isotopic determination of *n*-alkanes in diverse environmental samples that contain large amounts of unsaturated compounds. Furthermore, the off-line-based purification process that uses an Ag⁺-impregnated silica gel column appears to be well suited as a means to more accurately identify the isotopic compositions of individual *n*-alkanes in various types of environmental samples.

4 Conclusion

An off-line-based pretreatment method was assessed to provide an optimized approach for the isotopic analysis of *n*-alkanes present in various types of environmental samples. The use of the Ag⁺-impregnated silica column allowed the individual *n*-alkanes detected in each sample to be more effectively isolated from unsaturated compounds. Moreover, this approach (i.e., Method 2) yielded higher isotopic accuracy ($\pm 3.9\%$) than Method 1 ($\pm 7.4\%$) following GC–C–IRMS analysis. Thus, the Ag⁺-impregnated silica column separation method seems to effectively eliminate the possibility of interfering unsaturated compounds. Therefore, the Ag⁺-impregnated silica column can be practically applied for the $\delta^{13}\text{C}$ analysis of *n*-alkanes to increase the analytical precision of environmental sample measurements.

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