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Article

Compound-Specific $\delta^{13}C$ of *n*-Alkanes: Clean-Up Methods Appraisal and Application to Recent Sediments of a Highly Contaminated Bay

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Different clean-up methods to determine isotopic composition of *n*-alkanes were tested. Sources of organic matter in Guanabara Bay were re-examined through the $\delta^{13}C$ of individual *n*-alkanes in surface sediment samples. The *n*-alkanes were efficiently isolated without significant losses of the compounds of interest. The $\delta^{13}C$ of *n*-alkanes (*n*-C_{17} to *n*-C_{35}) ranged between –34.0 and –26.4‰. More depleted $\delta^{13}C$ values associated to higher carbon preference index (CPI > 4) suggest prevalence of biogenic *n*-alkanes from terrestrial origin in the inner stations influenced by inputs from mangrove areas. Small isotopic differences between odd and even-numbered chains and ^{13}C-enrichment in long-chain homologues indicate a petrogenic hydrocarbons contribution. Higher concentrations of short-chain compounds associated to a more ^{13}C-enriched total organic carbon (TOC) were found near the most intense sewage discharges. Results show that $\delta^{13}C$ of *n*-alkanes can be applied to a highly degraded system as the Guanabara Bay and help improve the characterization of the organic matter.

Keywords: GC-C-IRMS, UCM, urea adduction, organic matter, Guanabara Bay

Introduction

The composition and distribution of hydrocarbons in sediment samples provide information about the organic matter sources and transformation processes occurring in the environment.[1] Studies on *n*-alkanes have often been conducted to assess the origin of hydrocarbons, such as biogenic (marine or terrestrial) or petrogenic, found in the marine environment. The assessment makes use of the difference in the *n*-alkane chain length to draw conclusions on the sources of organic matter. In higher plants, for example, *n*-alkanes from epicuticular waxes show long chains and an odd over even-numbered carbon chain predominance.[2] Algae are characterized by shorter chain lengths with no expressive odd-over-even preference.[3] In contrast, *n*-alkanes in fossil fuels have a wide composition range, without predominance of odd or even-numbered chains.[4] However, despite these typical fingerprints, there are cases in which *n*-alkane profile of different classes of organisms overlap, resulting in ambiguous interpretations of the organic material sources.[5] Given this multiplicity of origins, an interpretation of the different sources exclusively by the concentrations of *n*-alkanes is very limited in more complex environments.[6]

During the last decades, the carbon isotopic analysis of some biomarkers have been widely used as a valuable tool to better distinguish the sources of organic matter in marine environments.[5,7-14] The carbon isotope composition in the biosynthesized organic material depends on both the isotopic composition of the carbon source used by organisms and the isotope discrimination that occurs during carbon fixation, which includes the steps of absorption and intracellular diffusion, and also the biosynthesis of the cell components.[15] Thus, when two classes of organisms present an overlap in the *n*-alkane distribution, additional information can be obtained from the specific compound isotopic composition.[10] The development of gas chromatography coupled to isotope ratio mass spectrometer via a combustion interface (GC-C-IRMS) has made possible the establishment of the compound-specific $\delta^{13}C$ of *n*-alkanes, which leads to a significant improvement regarding the characterization of these compounds sources.[6,16]

Differences between the $\delta^{13}C$ values of plant tissue are related to the isotopic fractionation during photosynthesis.[17] *n*-Alkanes are ca. 0 to 10‰ more ^{13}C-depleted than the bulk plant $\delta^{13}C$ values[7] due to the isotopic fractionation

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that occurs during the biosynthesis of these compounds. The *n*-alkanes are more ^{13}C-depleted in plants that use the C3 photosynthetic pathway (–31 to –39‰) and more ^{13}C-enriched for C4 plants (–18 to –25‰).[18] CAM (crassulacean acid metabolism) plants use both C3 and C4 carbon fixation pathways, so the $\delta^{13}C$ range are intermediate (−23 and −29‰).[10] Isotopic fractionation in photosynthetic marine organisms is more complex, being controlled by the concentration of dissolved CO_2, nutrients availability, pH and physiological factors such as cell size, growth rate, and membrane permeability.[19,20] The $\delta^{13}C$ values of aquatic plants are usually less negative than that of terrestrial plants.[21] In relation to petrogenic sources, the *n*-alkanes present in biodegraded oils are usually isotopically heavier than those biosynthesized by higher-plants.[16,22]

The present study aimed at improving the characterization of sources of organic matter present in sediments of the Guanabara Bay (Rio de Janeiro, RJ, Brazil) by using $\delta^{13}C$ fingerprinting of *n*-alkanes. To achieve accurate measurements of compound-specific $\delta^{13}C$ by GC-C-IRMS, a good performance during the separation of the chromatographic peaks is required.[23] Therefore, the isolation of the compounds of interest from an unresolved complex mixture (UCM) becomes essential. Previous studies using hydrocarbons before[24] and after the oil spill that occurred in 2000[25-29] show that Guanabara Bay is an extremely degraded environment. This results from the high population density and diversity of industrial activities in watershed, intense boat traffic and oil industry related activities. Because of these conditions, sediments from the bay commonly show a high content of UCM due to the presence of degraded oil, especially near the harbor.[27,30] Compounds in the UCM interferes with the determination of the $\delta^{13}C$ of *n*-alkanes, therefore, it is necessary to perform an effective clean-up of the extract. The clean-up must remove the branched and cyclic compounds that compose the UCM fraction and/or coelute with the peaks of interest in the chromatogram, without any losses in the analytical quality of minor compounds or isotopic fractionation during the separation process. To this end the performance of methods found in literature were evaluated.

Experimental

n-Alkanes isolation experiments

Methods obtained from the literature were tested and adjusted to isolate the *n*-alkanes without losses of analytical quality of the minor compounds or isotopic fractionation during the separation process. The tested methods were 5A molecular sieves[31,32] and urea adduction[33-36] to remove cyclic and branched compounds; and silica impregnated with $AgNO_3$ column for *n*-alkenes removal.[37] To evaluate quantitatively the possible losses that could occur from the various steps of each procedure, several experiments were conducted with a standard mixture containing the *n*-alkanes C_{12}-C_{40} and the isoprenoids pristane (Pr) and phytane (Ph) (AccuStandard, 500 μg mL^{-1}). Quantification was performed by gas chromatography with flame ionization detector (GC-FID) before and after the respective experiments based on the previous addition of internal standard *n*-C_{24}d and a calibration curve. To evaluate a possible isotopic fractionation in the *n*-alkanes during the procedure, the isolation methods that showed best recovery were applied to the *n*-alkanes standard mixture in triplicate and the standard was analyzed in the GC-C-IRMS before and after the procedure, following the conditions and quality assurance described below for the compound-specific $\delta^{13}C$ analyses.

Molecular sieves

The inclusion step was based on the methodology used by Tolosa and Ogrinc.[32] One gram of activated molecular sieve with the pore size of 5 Å and a bead size of 8-12 mesh (Sigma-Aldrich) was added to a reaction flask containing 20 μL of the standard mixture in about 2 mL of isooctane. The flask was left properly sealed overnight. After the inclusion, the isooctane solvent containing the branched and cyclic compounds was pipetted out and the sieves were washed 3 times with 2 mL of *n*-hexane, forming the excluded fraction (EX).

The extraction step was based on the methodology developed by Grice *et al.*,[31] which eliminates the use of hydrofluoric acid. After the sieves were completely dried at room temperature, 2 mL of cyclohexane/*n*-pentane (12%, v/v) were added to remove the *n*-alkanes included in the pores of the sieves. The reaction flask was properly sealed again and maintained at 80 °C for 8 h. The fraction containing the *n*-alkanes extracted from the sieves was transferred to a vial by washing the sieves 3 times with *n*-pentane, forming the included fraction (IN). Finally, the solvents of both fractions (IN and EX) were exchanged to *n*-hexane and concentrated to 1 mL under N_2 flow and the internal standard *n*-C_{24}d was added for quantification by GC-FID.

In order to obtain a higher recovery of *n*-alkanes, several conditions were tested, such as: the molecular sieves surface area, the use of ultrasound to remove the EX fraction, and the temperature during the inclusion and extraction steps (Table 1). The different conditions were modified according to the recovery obtained for the compounds in each experiment for the IN and EX fractions.

Table 1. Different conditions tested for the molecular sieves method

Experiment	Sieves size	Temperature of inclusion / °C	Sieves wash by ultrasound	Temperature of extraction / °C
A	8-12 mesh	25	no	80
B	8-12 mesh	90	no	80
C	8-12 mesh	90	yes	75
D	8-12 mesh	90	yes	80
E	broken	90	yes	80
F	8-12 mesh	25	yes	68, 80, 85
G	broken	25	yes	68, 80, 85

Urea adduction

The solvents *n*-hexane, acetone and a solution of urea in methanol were slowly and carefully added to a vial containing 20 µL of the standard mixture to form the urea adduct. For being the most critical step of the procedure, different conditions were tested for the crystal formation, such as the volume of *n*-hexane and urea solution, concentration of the urea solution, stirring and temperature (Table 2).

After complete formation of the urea crystals, all experiments followed the same procedure. The solvents were evaporated under N_2 stream and the crystals washed 3 times with *n*-hexane to remove the branched and cyclic non-adducted compounds. To recover the adducted *n*-alkanes, the crystals were dissolved in water and the compounds were extracted 3 times with *n*-hexane. Both adducted and non-adducted fractions were concentrated to 1 mL under N_2 flow and the internal standard *n*-C_{24}d was added for quantification by GC-FID. The efficiency of *n*-alkanes recovery in water was tested in an isolated experiment by making a standard mixture solution with water and quantifying the three successive *n*-hexane extractions separately.

Silica impregnated with $AgNO_3$

For the preparation of silica gel impregnated with $AgNO_3$ (10 wt.%), 1 g of $AgNO_3$ was diluted in ethanol and water and mixed with 9 g of silica gel. The solvents were rotary evaporated and the silica activated at 100 °C for 2 h. A Pasteur pipette (5 mm i.d.) was packed with 1 cm of $AgNO_3$-silica gel (10 wt.%) and the saturated hydrocarbon fractions of the standard mixture was eluted with 4 mL hexane, following the procedure suggested by Albergaria-Barbosa (personal communication).[38] This fraction was concentrated to 1 mL under N_2 flow and quantified by GC-FID after addition of internal standard *n*-C_{24}d.

Study area and sampling

The Guanabara Bay, located in the state of Rio de Janeiro, is amongst the largest bays of the Brazilian coast, with an area of 384 km^2. Its hydrographic basin covers an area of about 4,080 km^2 occupied by approximately 10 million inhabitants.[39,40] It is considered one of the most degraded ecosystems of the Brazilian coast.[41] Several studies conducted in Guanabara Bay show environmental impacts such as eutrophication,[42,43] high sedimentation rates,[44,45] high concentrations of metals,[46-51] and hydrocarbons[25,27,30,52,53] in water, sediment and biota. Among the main sources of pollution to the bay, stand out more than 14,000 industries, domestic sewage discharges with low or no treatment, 14 oil terminals, 2 harbors, 32 boatyards and more than 1000 gas stations.[54]

Table 2. Different conditions tested for the urea adduction method

Experiment	*n*-Hexane / µL	Acetone / µL	Urea solution / µL	Duration of stirring + standby / min	Temperature of crystal formation / °C	Duration of crystal formation / min
A	200	200	200 (10%)	–	–4	30
B	200	200	200 (10%)	–	0	30
C	200	200	200 (10%)	–	room temperature	overnight
D	200	200	200 (10%)	overnight + 90	–4	30
E	200	200	400 (10%)	–	–4	30
F	200	200	200 (saturated)	–	–4	30
G	400	200	200 (saturated)	30 + 90	–4	30

There are 55 rivers flowing into the bay and together they are responsible for a mean annual discharge of about 351 m^3 s^{-1}.[39] Except in the central channel, most of the bay is composed by fine sediments like clay and silt[40] and contains high levels of organic matter, especially in the west and northwest areas where the rivers are heavily polluted by domestic and industrial residues.[55] Mangrove forests surrounding the bay have an area of about 81 km^2 and the largest part is concentrated in the environmental protection area. This area represents about 31% of the original forest and the main factors responsible for its degradation are the construction of embankments, urbanization and industrial expansion.[55]

Samples were collected according to Massone *et al.*[27] in order to cover a wide area taking into consideration the activities carried out in the bay. In brief, eleven different sites were selected in the Guanabara Bay (Figure 1) and surface sediments (2 cm) were sampled in April 2012 using a modified Van Veen sampler as to avoid the washing out of fine surface layers.

Sediment samples clean-up

The isolation procedures that showed best results were applied to the aliphatic hydrocarbon fractions of the Guanabara Bay sediments prior to compound-specific $\delta^{13}C$ analysis. The samples were re-injected into the GC-FID for quantification to verify the efficiency of UCM removal and the possible losses that may have been caused by each method. The clean-up procedure was repeated for the samples that still exhibited interfering compounds.

Aliphatic hydrocarbons quantification

The quantification of aliphatic compounds was based on EPA 8015C method.[56] The dry sediment samples (10 g) were extracted with dichloromethane using an accelerated solvent extraction system (ASE 200, Dionex). The conditions were: 80 °C, 1800 psi, 8 min extraction, 60 s N_2 purge and two extraction cycles. Before the extraction, 2500 ng of the surrogate standard *n*-C_{30}d was

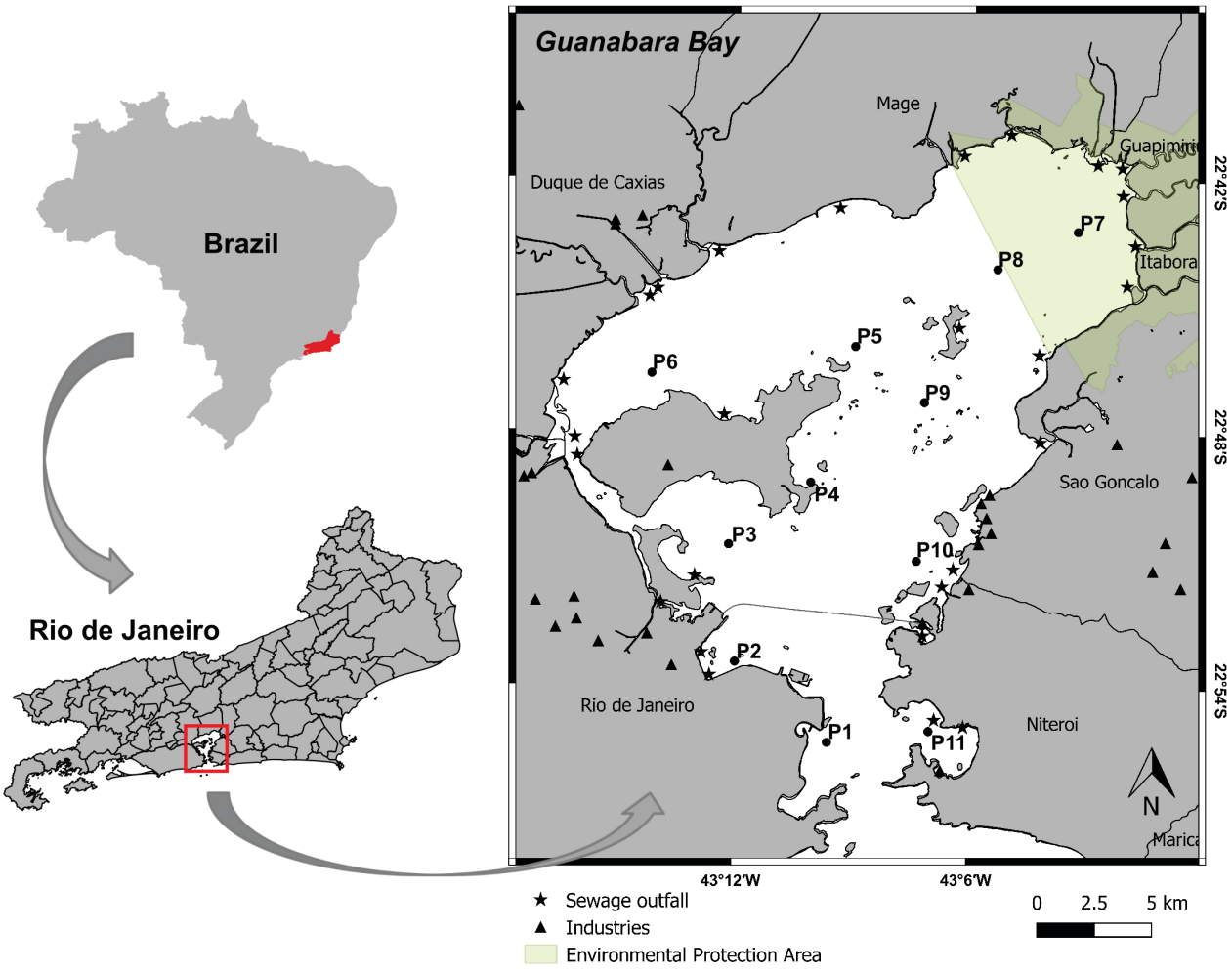

Figure 1. Guanabara Bay sampling sites.

added to evaluate the recoveries. Extract volume was reduced under N_2 stream and the solvent was exchanged to *n*-hexane.

The hydrocarbons fractions were separated in a glass column (1.3 cm i.d. and 30 cm height) packed with 1 cm of copper, 1 g of anhydrous Na_2SO_4, 7 g of alumina (2% water deactivated) and 10 g of silica. The aliphatic fraction was eluted with 60 mL of *n*-hexane, the extract was concentrated to 1 mL under N_2 stream and the internal standard *n*-C_{24}d was added to the fraction.

n-Alkanes from *n*-C_{12} to *n*-C_{40}, the isoprenoids pristane and phytane, the UCM and the resolved peaks (RP) were determined by GC-FID equipped with a DB-5 column (Agilent, 30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was the carrier gas (2 mL min^{-1}, 5 psi), nitrogen the makeup gas (33 mL min^{-1}), air (360 mL min^{-1}) and hydrogen (33 mL min^{-1}) were used in the detector. Injector and detector temperatures were 290 and 310 °C, respectively. Oven temperature was programmed as follows: initial hold at 50 °C (0.75 min), increase at 20 °C min^{-1} up to 80 °C, followed by 6 °C min^{-1} up to 310 °C (20 min). The retention times were determined using a standard solution containing all compounds of interest and quantifications were based on internal standard addition and calibration curves (9 standard solutions, from 0.05 to 50 μg mL^{-1}). UCM is the difference between the total area of aliphatic fraction and the RP areas integrated. The limit of detection was determined by injecting 8 replicates of a standard solution (C_{12}-C_{40}) and multiplying the standard deviation for 3. The limit of quantification is the lowest concentration in the calibration curve. Limits of detection and quantification for *n*-alkanes were 2.5 and 5 ng g^{-1}, respectively. The limit of quantification for the UCM was 145 ng g^{-1} and consists of the sum of all the compounds in the lowest concentration of the calibration curve. Average recovery for *n*-C_{30}d standard was 104 ± 12%. To verify the accuracy of the method, the certified reference material NIST SRM 1944 was analyzed in duplicate. A blank was also analyzed and its results were subtracted from the samples results.

Compound-specific $\delta^{13}C$ analyses

The $\delta^{13}C$ values of *n*-alkanes were determined using Trace GC Ultra gas chromatograph coupled to a Delta V Plus isotope ratio mass spectrometer via a GC Isolink combustion (Thermo). The chromatograph was equipped with a DB-5 column (30 m × 0.25 mm i.d. × 0.25 μm film thickness), helium was used as carrier gas (1.5 mL min^{-1}), injector and detector temperatures were of 250 and 310 °C, respectively, and oven temperature was programmed as follows: initial hold at 50 °C (0.75 min), increase at 20 °C min^{-1} up to 120 °C, followed by 4 °C min^{-1} up to 310 °C (15 min).

The results are expressed as *per* mil (‰) relative to the CO_2 reference gas, which is previously calibrated relative to the $\delta^{13}C$ of Vienna Pee Dee Belemnite (VPDB). At the beginning and the end of each analysis, three pulses of the reference gas (CO_2) were introduced with the intensity of 3000 mV. The isotopic ratio of each *n*-alkane peak was calculated according to the value of the third pulse. The $\delta^{13}C$ values are reported as the average of two injections for peaks higher than 500 mV and as the average of four injections for peaks between 200 and 500 mV. Only values that showed a standard deviation of less than 0.5‰ were accepted.

An external standard consisting of *n*-alkanes (*n*-C_{16} to *n*-C_{30}) of known isotopic compositions acquired from Indiana University (USA) was injected between samples to ensure the accuracy of the analysis (deviation below ± 0.5‰ for all the compounds). The $\delta^{13}C$ of the surrogate standard *n*-C_{30}d and of the internal standard *n*-C_{24}d were also analyzed in the samples and compared with the $\delta^{13}C$ previously determined in the GC-C-IRMS prior to any treatment (*n*-C_{30}d = –33.0 ± 0.23‰ and *n*-C_{24}d = –32.5 ± 0.11‰) to verify if isotopic fractionation during the sample preparation occurs.

Total organic carbon $\delta^{13}C$ analyses

Dry sediment samples were weighed (0.2 mg) in silver capsules and acidified with HCl solution (1 mol L^{-1}) to remove carbonate. $\delta^{13}C$ of total organic carbon (TOC) was determined using Flash elemental analyzer coupled to Delta V isotope ratio mass spectrometer (Thermo). At the beginning of each analysis, three pulses of the reference gas (CO_2) were introduced with an intensity of 8000 mV. The $\delta^{13}C$ was calculated according to the value of the third pulse. The gas was previously calibrated with the IAEA USGS40 standard material.

Results and Discussion

n-Alkanes isolation experiments

The results obtained for the experiments with the molecular sieves are shown in Table 3. The temperature of 90 °C was chosen for the inclusion step in some experiments because it was the highest temperature in which the solvent did not evaporate. Experiments A and B showed small amounts of pristane and phytane in the included fraction. Given that they are not included because of the branched

chains, these compounds were probably still attached to the surface of the sieves, requiring a more efficient washing with *n*-hexane to completely remove the excluded fraction. Therefore, in the subsequent experiments, the excluded fraction was removed by washing the sieves 3 times with 2 mL of *n*-hexane by ultrasonication for 5 min.[32]

A solvent bubbling was observed in the extraction step at 80 °C and this may have contributed to the lighter compounds loss. The extraction temperature was reduced to 75 °C in experiment C and the results were better (> 50% from *n*-C_{16} to *n*-C_{25}), but bubbling was still observed and heavier compounds were not extracted.

Suspecting that the solvent bubbling could assist in the extraction of heavier compounds within the pores, experiments D and E were repeated at 80 °C. The difference between the procedures was the surface area of the sieves, increased in experiment E by breaking the sieves in smaller pieces. Both experiments showed unsatisfactory results with very low recovery of the *n*-alkanes. The losses could be related to the bubbling, sealing problems of the reaction flask or compounds remaining in the pores of the sieves. It was also observed a low recovery of the compounds pristane and phytane in the excluded fraction, which indicates losses in the high temperature inclusion step.

To test the best temperature for the *n*-alkanes removal from the sieves pores, experiments F and G were conducted by repeating the process three times, increasing the extraction temperature. The inclusion step was carried out at room temperature with a higher surface area for the sieves in experiment G. The first temperature chosen was 68 °C, the maximum temperature at which no bubbling was observed for the solvents. The first excluded fraction

Table 3. Recovery of the compounds of a standard mixture of *n*-alkanes after isolation tests with molecular sieves 5A

Compound	A / %	B / %	C / %	D / %	E / %	F / %	G / %
C_{12}	3.65	24.79	36.01	26.03	2.68	43.69	21.82
C_{13}	4.23	25.59	39.50	30.79	2.31	48.31	31.92
C_{14}	6.43	27.28	43.05	32.35	1.97	52.51	41.11
C_{15}	8.71	29.79	47.30	33.29	1.22	53.95	45.17
C_{16}	9.84	34.42	53.92	35.19	2.34	58.63	51.61
C_{17}	11.24	38.00	60.13	36.54	2.33	61.93	54.43
Pristane	5.73	12.62	4.77	3.81	7.71	3.38	7.84
C_{18}	12.48	38.56	60.95	33.27	2.03	63.00	51.54
Phytane	6.76	14.76	6.23	4.61	8.60	3.57	8.52
C_{19}	13.95	37.22	60.04	30.62	1.08	70.94	47.01
C_{20}	17.21	35.83	58.55	30.26	0.78	81.83	46.54
C_{21}	23.04	35.92	58.90	31.44	0.14	88.87	46.44
C_{22}	29.10	36.43	60.31	33.69	0.00	89.57	45.03
C_{23}	31.45	35.33	59.67	33.61	0.00	90.74	42.71
C_{24}	31.55	32.26	57.40	30.31	0.15	88.61	37.84
C_{25}	29.89	29.58	51.79	27.85	0.46	83.41	35.31
C_{26}	26.75	26.25	45.61	24.56	0.46	74.61	29.59
C_{27}	25.05	24.70	41.86	23.19	1.08	69.67	27.13
C_{28}	22.89	21.41	36.45	20.13	0.74	58.68	21.84
C_{29}	19.10	18.52	31.87	17.10	0.62	48.62	17.94
C_{30}	16.03	14.46	26.34	13.91	0.78	38.04	15.40
C_{31}	14.01	12.25	23.07	11.57	1.03	31.51	13.60
C_{32}	12.40	10.15	19.87	9.66	1.11	25.29	11.61
C_{33}	11.51	8.77	17.38	7.64	1.84	21.30	11.82
C_{34}	10.08	7.23	14.64	6.15	3.17	17.91	13.38
C_{35}	10.25	6.34	12.73	4.87	5.55	17.21	17.93
C_{36}	9.55	4.65	9.51	3.16	7.10	14.19	20.60
C_{37}	10.05	4.36	8.42	2.97	10.37	16.58	27.75
C_{38}	9.66	4.02	6.87	2.55	13.00	17.96	33.17
C_{39}	9.63	5.88	6.65	3.36	15.61	26.20	46.25
C_{40}	8.88	3.98	5.52	2.36	16.68	20.47	38.74

Recovery was < 20% for all compounds in experiment E; < 40% for all compounds in experiments A, B and D; < 61% for all compounds in experiments C and G. Experiment F showed the best results, with recovery > 80% from *n*-C_{20} to *n*-C_{25}. Overall, highest recoveries occurred from *n*-C_{17} to *n*-C_{28}. Pristane and phytane showed low recoveries for all the experiments because they are not included in the pores of molecular sieves 5A.

was collected and another 2 mL of cyclohexane/*n*-pentane (12%, v/v) was added. The second temperature chosen was 80 °C, suggested by Grice *et al*.[31] The extraction process was repeated for the third time, with the temperature of 85 °C, used by Sun *et al*.[57] to adapt the same methodology. Combining the three fractions, experiment F showed the best results with a considerable increase in the recovery of compounds. The recovery was > 80% from *n*-C_{20} to *n*-C_{25}, but < 60% from *n*-C_{12} to *n*-C_{16}, between 30 and 80% from *n*-C_{26} to *n*-C_{31} and < 30% from *n*-C_{32} to *n*-C_{40}. It is important to notice that experiments E and G showed higher concentrations of pristane and phytane than D and F, which means that an increase in the surface area of the sieves could avoid the removal of the excluded fraction.

Even though experiment F showed the highest recovery among the tested procedures, the molecular sieves method cannot be applied to the sediment samples under our conditions because significant losses were still observed for the compounds of interest, especially those < *n*-C_{20} and > *n*-C_{30}. An explanation is the possible loss of the short-chain *n*-alkanes during solvent bubbling in the high temperature extraction associated with the incomplete removal of the long-chain compounds from the sieve pores.

Results for the tests with the urea adduct are shown in Table 4. Initially, the tests followed the procedure suggested by Canuel (personal communication),[58] in which the crystal formation is carried out at low temperature to recover the low molecular weight (LMW) *n*-alkanes.[34] It was observed that all the compounds, especially the LMW *n*-alkanes, appeared in the non-adducted fraction, which means they were not completely adducted by the crystals. To verify if the –4 °C cooling was responsible for this low efficiency in

Table 4. Recovery of the compounds of a standard mixture of *n*-alkanes after the isolation experiments with urea adduction

Compound	A / %	B / %	C / %	D / %	E / %	F / %	G / %
C_{12}	12.33	10.57	24.03	19.92	23.74	41.76	69.74
C_{13}	19.24	15.99	29.86	23.46	35.40	54.31	74.69
C_{14}	23.49	19.90	33.53	26.22	39.74	60.80	75.74
C_{15}	19.39	16.02	28.48	20.98	32.88	63.78	76.37
C_{16}	26.90	24.41	34.03	27.84	37.62	65.44	76.97
C_{17}	26.14	24.81	35.01	29.00	20.78	67.16	79.14
Pristane	1.53	1.13	2.84	2.29	19.27	20.38	–
C_{18}	26.25	25.19	35.68	29.29	37.88	69.39	78.90
Phytane	1.87	1.55	3.20	2.58	15.85	21.07	–
C_{19}	25.75	25.44	38.81	30.11	35.68	72.59	81.58
C_{20}	31.13	28.14	44.09	34.41	45.74	77.22	87.39
C_{21}	34.63	33.58	48.52	36.94	48.77	79.81	91.16
C_{22}	35.53	33.56	48.67	36.30	49.65	81.24	92.85
C_{23}	35.44	33.62	49.40	36.28	50.76	81.75	93.71
C_{24}	35.14	33.48	49.17	36.44	50.78	82.45	93.81
C_{25}	35.17	33.87	49.08	36.60	51.16	83.07	93.64
C_{26}	35.23	33.77	49.32	36.74	51.68	83.21	93.81
C_{27}	33.26	32.43	51.13	37.17	53.51	83.30	93.83
C_{28}	33.97	32.92	48.68	35.76	51.26	83.50	93.56
C_{29}	33.38	32.43	48.49	34.97	51.00	83.68	93.89
C_{30}	32.97	32.00	48.00	34.75	50.07	83.66	93.67
C_{31}	31.57	30.24	47.01	33.78	48.27	83.74	93.95
C_{32}	30.89	29.72	46.02	32.96	46.62	83.76	93.80
C_{33}	29.63	29.14	45.02	32.36	45.80	83.70	93.94
C_{34}	27.72	26.66	41.61	30.19	43.02	83.29	93.58
C_{35}	26.15	25.38	40.36	29.67	40.40	83.59	94.33
C_{36}	21.83	21.88	36.56	26.78	38.28	83.39	94.38
C_{37}	19.42	18.95	34.02	25.98	36.62	83.50	94.50
C_{38}	17.58	17.51	30.56	25.61	35.60	83.13	95.49
C_{39}	13.57	14.09	26.36	23.80	33.99	82.76	93.55
C_{40}	11.99	11.59	23.43	23.59	32.52	82.73	95.49

Recovery was < 40% for all compounds in experiments A, B and D; < 60% for all compounds in experiments D and E; < 61% for all compounds in experiments C and G. Experiment F showed recovery > 80% from *n*-C_{22} to *n*-C_{40}. After small adjustments, experiment G showed better results, with recoveries > 90% from *n*-C_{22} to *n*-C_{40}; > 80% for *n*-C_{19} and *n*-C_{20}; and > 70% from *n*-C_{13} to *n*-C_{18}. Overall, highest recoveries occurred from *n*-C_{20} to *n*-C_{35}. Pristane and phytane showed low recoveries for all the experiments because they are not included in the urea adduct channels.

the paraffins inclusion, experiment B was performed at 0 °C (ice bath) for 30 min, and experiment C at room temperature for 24 h.[33] However, the results were unsatisfactory and similar to experiment A for both.

According to Nwadinigwe and Nwobodo,[35] a mixing process ensures that there is an effective contact between the urea and *n*-paraffins and, therefore, is very useful in the separation of compounds that form adduct from the cyclic and branched compounds. Experiment D was performed based on this principle, and the solution was left stirring for 24 h,[36] followed by 1 h and 30 min standing at room temperature and 30 min at –4 °C.

A low recovery of compounds was also observed for experiment D, so experiment E was developed by adding twice the volume of urea solution (10%) and removing the stirring step. The results for this experiment were considerably better than the previous ones, indicating that the excess of urea could be crucial for an efficient inclusion of compounds within the crystals. According to Nwadinigwe and Nwobodo,[35] the formation of the urea complex is an equilibrium process, therefore the use of a large excess of urea would assist the process.

To ensure the urea excess, the following tests were made with a saturated urea solution in methanol.[33,36] Experiment F was carried out in the same conditions of experiment A, while in experiment G a stirring period of 30 min was added, followed by 1 h and 30 min standing at room temperature and 30 min at –4 °C. Another difference between these two experiments is the volume of *n*-hexane, which changed from 200 to 400 μL in experiment G. Experiments F and G presented a considerable increase in the recovery for compounds > *n*-C_{16}, which confirm the hypothesis that the excess of urea could be essential to improve the inclusion process within the crystals. Experiment G showed better results for all compounds, with a recovery > 69% from *n*-C_{12} to *n*-C_{18}, > 80% from *n*-C_{19} to *n*-C_{20} and > 90% from *n*-C_{21} to *n*-C_{40}. Besides having quantitatively satisfactory results, the urea adduction method showed no isotopic fractionation when analyzed by GC-C-IRMS, except for *n*-C_{40}.

As in the tests with the molecular sieves, pristane and phytane appear in low concentrations due to the presence of branches in their structure, which prevents them from being included in the urea crystals. In relation to the isolated extraction step, the first extraction was responsible for 100% of the compounds > *n*-C_{24} recovery. On the other hand, even that the most part of the lighter compounds are also removed in the first extraction, they are still present in the second and third, probably because of a slightly higher affinity for water.

The silica impregnated with $AgNO_3$ (10 wt.%) column showed recovery > 90% for all compounds, except for *n*-C_{40} with 89%. This means that the procedure does not show expressive loss of compounds of interest.

Based on the recovery results obtained from all the experiments, a combination of $AgNO_3$-silica gel column to remove the unsaturated compounds and urea adduction (experiment G) to remove the cyclic and branched compounds was defined as the most suitable procedure to isolate the *n*-alkanes without considerable loss of the compounds of interest. The $\delta^{13}C$ results for the *n*-alkanes standard mixture before and after this procedure showed no fractionation for the *n*-alkanes from *n*-C_{14} to *n*-C_{39} (deviation below ± 0.5‰ between the average results of the triplicate analyzed after the procedure and the results prior to the treatment). *n*-C_{12} and *n*-C_{13} were not evaluated in this test. *n*-C_{40} showed a deviation of –1.3‰.

Aliphatic hydrocarbons

Results for the aliphatic hydrocarbons are shown in Table 5. Total aliphatic hydrocarbons ranged between 8 and 790 μg g^{-1}. Stations P1, P7, P10 and P11 (Figure 1) showed the lowest concentrations (< 100 μg g^{-1}). P1, P10 and P11 are located near the entrance of the bay, where the renewal of the water is faster due to the strong currents of the central channel. P7 is located in the inner eastern portion of the bay, inside an environmental protection area that represents the less degraded region of the bay. The highest concentrations were observed for the stations in the west side of the bay, characterized by a more intense boat traffic and a higher input of domestic sewage without treatment. In a recent study with chemical and biological indicators of sewage input, Costa *et al*.[41] observed that rivers in the western sector of the Guanabara Bay receive a more expressive amount of suspended material from organic inputs from the watershed in relation to the northeast rivers.

UCM concentrations varied between below the limit of detection for station P7, the least contaminated area of the bay, and 754 μg g^{-1} for P2, located near the Rio de Janeiro harbor area, where boat traffic is intense. Contamination by degraded oil can be analyzed through the relationship between UCM and the RP, where values of UCM/RP > 4 indicate biodegradation of oil derived compounds.[59] Results below this value may be a sign of recent contamination or low presence of petrogenic hydrocarbons in the environment.[60] UCM/RP > 4 were found for most stations, except for P1, P7 and P10. For all samples, except the P7 station, UCM constitutes 73-95% of the total aliphatic hydrocarbons. These results indicate a high degree of degradation of hydrocarbons of the aliphatic fraction in sediments of Guanabara Bay and a higher level of oil contamination in the western portion of the bay.

Table 5. Aliphatic hydrocarbons ratios in surface sediments from Guanabara Bay

	n-Alkane / (μg g^{-1})	Total aliphatic / (μg g^{-1})	UCM[a] / (μg g^{-1})	CPI[b]	ACL[c]	TAR[d]	C_{17}/Pr[e]	C_{18}/Ph[f]	UCM/RP[g]	UCM/∑*n*-alkanes
P1	0.89	10.57	7.72	3.16	25.66	1.92	1.31	1.39	2.71	8.71
P2	7.76	790.14	754.37	2.74	29.75	7.59	0.54	0.24	21.09	97.23
P3	6.00	431.96	408.36	2.59	28.42	9.14	1.26	0.68	17.30	68.06
P4	2.34	101.32	92.29	3.40	27.09	3.32	1.00	0.89	10.21	39.52
P5	5.88	205.63	187.72	3.79	26.68	4.59	1.91	1.40	10.48	31.93
P6	9.64	478.71	449.79	4.68	29.38	27.37	1.03	0.39	15.55	46.68
P7	11.94	10.89	< LOQ	4.94	28.61	185.85	1.36	0.52	–	–
P8	12.65	109.65	91.35	4.68	28.91	63.68	2.18	0.77	4.99	7.22
P9	3.57	335.62	317.38	3.16	28.78	12.76	1.06	0.52	17.40	88.87
P10	0.60	8.24	6.35	2.37	29.14	10.99	0.67	0.44	3.37	10.67
P11	2.04	88.48	81.10	3.10	29.16	13.23	0.64	0.42	10.99	39.69

[a]Unresolved complex mixture; [b]carbon preference index; [c]average chain length; [d]terrigenous/aquatic ratio; [e]pristane; [f]phytane; [g]resolved peaks. LOQ: limit of quantification.

As the UCM, pristane and phytane isoprenoids are also more resistant to degradation, so their concentration in respect to more labile *n*-alkanes like *n*-C_{17} and *n*-C_{18} can be used as an indicator of this process.[61] More precisely, these indices have been used as indicators of oil biodegradation.[62-64] As expected, the lowest values for the C_{17}/Pr and C_{18}/Ph ratios were observed at P2, which suggest a higher level of oil biodegradation near the harbor. Low values were also found for P10 and P11, the same stations that presented small concentrations of total *n*-alkanes and UCM. Unlike P2, these two stations do not show evidence of oil contamination and the low C_{17}/Pr and C_{18}/Ph are possibly related to a relatively higher degradation caused by the oxygenation of the fast-renewed waters. Alternatively, the low C_{17}/Pr and C_{18}/Ph can be result of a higher input of pristane and phytane. According to Ten Haven *et al.*,[65] the interpretation of these ratios sometimes may not be reliable due to the wide variety of sources for these isoprenoid compounds.

Comparing these results with other degraded environments,[7,66-69] it is observed that, although these samples exhibit intermediate values for the total *n*-alkanes, UCM concentrations are higher than in most other regions. Significantly higher concentrations of UCM were found by Wagener *et al.*[30] near the station P2, which may indicate heterogeneity of the bay sediments that can be explained by the action of the tides in the remobilization and transport or even by dredging activities and sediment transport to outside the bay.

The carbon preference index (CPI) gives the relative proportion of odd and even carbon number *n*-alkanes.[70] CPI (*n*-C_{24} to *n*-C_{34}) values ranged between 2.4 and 4.9. CPI values between 5 and 7 indicate a predominance of odd chains and can be related to fresh terrestrial material.[71] Values close to 5 were found only for stations P6, P7 and P8, located in the innermost region of the bay. Significantly higher values of the terrigenous/aquatic ratio (TAR) were found for these stations, especially for P7 and P8, stations nearby the environmental protection area of the bay. According to Bourbonniere and Meyers,[72] these values indicate a greater influence of organic matter from land sources in relation to aquatic sources in this region. CPI values around 1 are associated with contamination by petrogenic hydrocarbons, since they have no predominance of either odd or even-numbered carbon chains.[61,73] For the other stations values are lower, but not close to 1, and could be related to a mixture of biogenic and petrogenic sources for most of the bay.

The average chain length (ACL) of the *n*-alkanes is a proxy calculated using the concentration of the compounds from *n*-C_{14} to *n*-C_{34} and is based on the fact that terrestrial sources produce longer chains, which allows its use in characterizing the contribution of allochthonous organic matter.[5,74] The average value of the ACL was 28.3 ± 1.3 and represents predominance of longer chain alkanes for most stations, which may be a result of the degradation of the lighter compounds and a preferred conservation of the heaviest. Most of the stations showed values > 28 suggesting a strong terrestrial influence on the bay. The lowest ACL were found for the stations P1, P4 and P5 and indicate a larger influence of marine inputs to these stations in relation to the others. It is possible to note the presence

of homologous series of LMW for these stations (Figure 2). This greater relative contribution of lighter compounds, compared to other samples may be related to a greater input of nutrients from the nearby sewage discharges, leading to intense algae blooms. However, despite being an eutrophic environment[40] with a high primary production,[75] n-C_{17}/n-C_{29} ratios are low for all stations (average of 0.11), probably due to a greater lability of LMW *n*-alkanes during the degradation processes associated with the contribution from terrestrial organic matter. With this, a more specific interpretation of these results becomes necessary and could be achieved by determining the stable carbon isotopic ratio of the individual *n*-alkanes.

Sediment samples clean-up

The branched and cyclic compounds were efficiently removed from each sample via $AgNO_3$silica gel column followed by urea adduction. The UCM removal was > 90% for all sediment samples. Figure 3 shows as an example the chromatograms obtained for the aliphatic fraction of the sample P5 before and after clean-up. It can be observed that almost the entire unresolved fraction was removed and the *n*-alkanes of interest were isolated from the aliphatic fraction without apparent losses. The chromatogram after the $AgNO_3$-silica gel column (Figure 3b) shows almost no difference from the chromatogram before the procedure

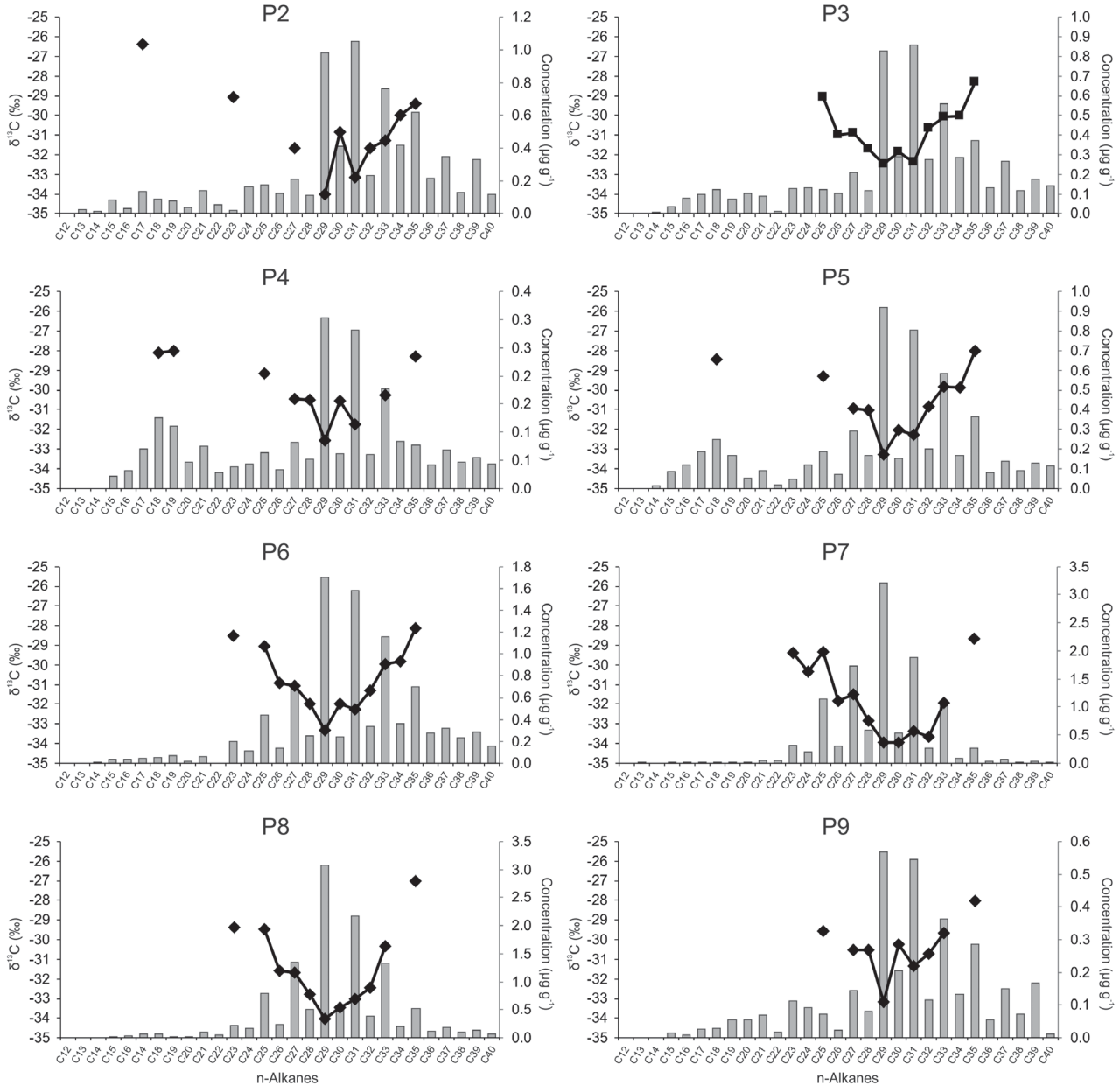

Figure 2. Concentration (µg g^{-1}) and $\delta^{13}C$ (‰) of *n*-alkanes (C_{16}-C_{40}) in Guanabara Bay sediment samples.

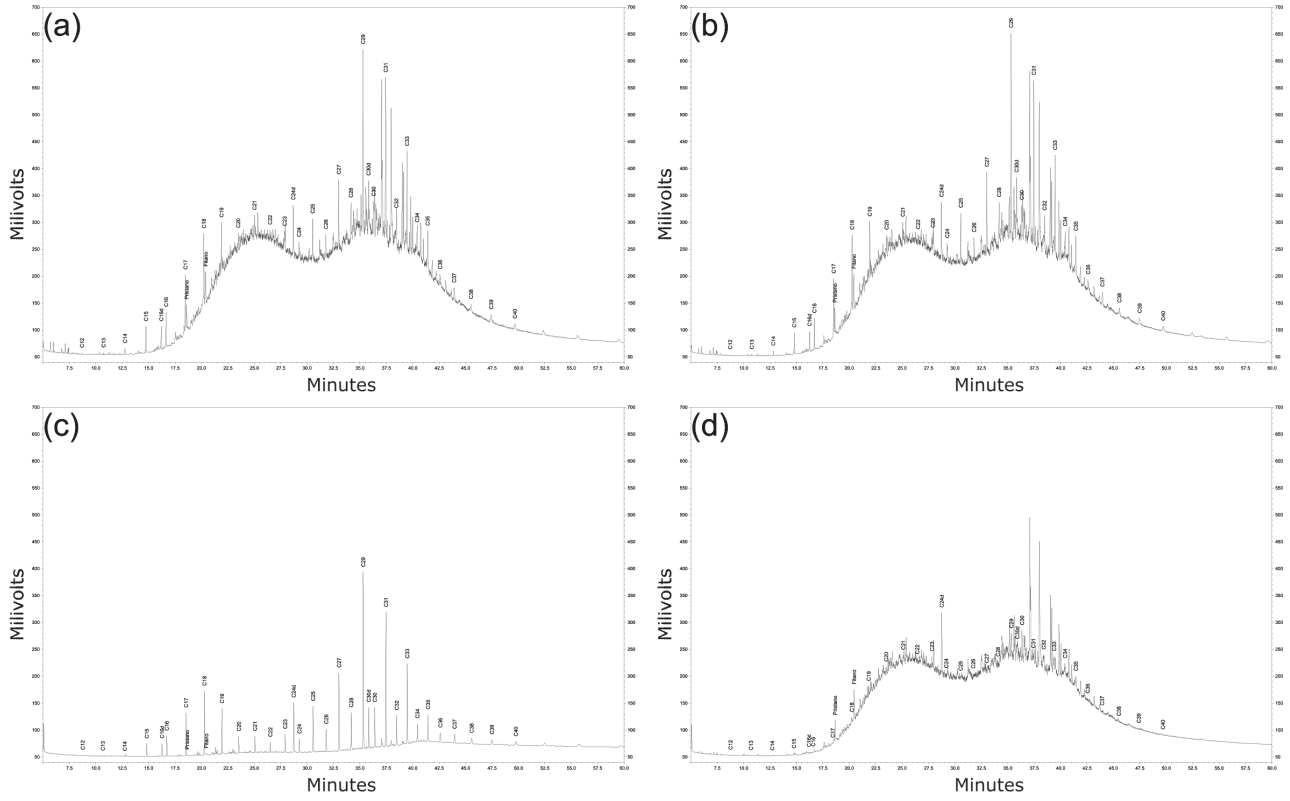

Figure 3. Gas chromatograms of aliphatic hydrocarbons from sample P5 (a) before *n*-alkane isolation; (b) after removal of the *n*-alkenes with silica-$AgNO_3$ column; (c) after removal of cyclic and branched compounds with urea adduction and (d) no*n*-adducted fraction.

and the UCM is efficiently removed only after the urea adduction (Figure 3c). This happens because the UCM is mainly composed by branched and cyclic compounds, which are not included inside the hexagonal channel structures of the urea adduct. However, unsaturated compounds such as *n*-alkenes are included together with the *n*-alkanes in the formation of the urea adduct. Thus, even though is not apparent in the chromatogram, the $AgNO_3$-silica gel column is essential for the removal of *n*-alkenes that cannot be removed by urea adduction and might be coeluting with the *n*-alkanes.

$\delta^{13}C$ of *n*-alkanes and TOC

The $\delta^{13}C$ values for *n*-alkanes and TOC detected in Guanabara Bay sediment samples are shown in Table 6. Samples P1, P10 and P11 were not analyzed because they showed very low concentrations for the *n*-alkanes of interest. Most of the short-chain *n*-alkanes (*n*-C_{17} to *n*-C_{24}) were not determined for being below the limit of quantification. The $\delta^{13}C$ signatures varied between –34.0 and –26.4‰ for the analyzed *n*-alkanes (*n*-C_{17} to *n*-C_{35}), suggesting a mixture of sources for the sediments of the bay, and is in the same range of other contaminated environments.[7,13,16,76,77] The mean standard deviation for all determinations was 0.21‰. The $\delta^{13}C$ of the surrogate and internal standard added to the samples were also determined (except for *n*-C_{30}d in P5 and P8) and the results did not show variation between samples that could represent any isotopic fractionation during both the extraction and separation processes of the aliphatic fraction ($\delta^{13}C$ = –33.1 ± 0.26‰ for *n*-C_{30}d), and for the *n*-alkanes isolation step ($\delta^{13}C$ = –32.8 ± 0.22‰ for *n*-C_{24}d).

The plotted $\delta^{13}C$ signatures in Figure 2 shows that the $\delta^{13}C$ values become more depleted with increasing carbon number up to *n*-C_{31}, a pattern commonly observed in other studies.[5,7,78] Overall, the short-chain *n*-alkanes (*n*-C_{17} to *n*-C_{24}) had values in the range of –30.4 to –26.4‰, while the long-chain (*n*-C_{25} to *n*-C_{35}) were between –34.0 and –27.0‰. The short-chain *n*-alkanes results are scarce and less representative of the entire set of samples in relation to the long-chain *n*-alkanes, but still provide a good example of the isotopic differences between LMW and high molecular weight compounds (HMW). The LMW homologues are more enriched in ^{13}C as they are associated with phytoplankton. As for the HMW, they can be derived from the terrestrial plants contribution and oil contamination.

In general, HMW compounds are more ^{13}C-depleted and may be associated with a contribution from C3 plants.[18,79,80] The $\delta^{13}C$ most depleted values for *n*-C_{27}, *n*-C_{29} and *n*-C_{31}

Table 6. $\delta^{13}C$ of *n*-alkanes (*n*-C_{17} to *n*-C_{35}) in Guanabara Bay sediment samples

n-Alkane	P2 / ‰	P3 / ‰	P4 / ‰	P5 / ‰	P6 / ‰	P7 / ‰	P8 / ‰	P9 / ‰
C_{17}	–26.3 ± 0.3[a]	nd	nd	nd	nd	nd	nd	nd
C_{18}	nd	nd	–28.1 ± 0.1	–28.4 ± 0.005	nd	nd	nd	nd
C_{19}	nd	nd	–28.0 ± 0.02	nd	nd	nd	nd	nd
C_{20}	nd	nd	nd	nd	nd	nd	nd	nd
C_{21}	nd	nd	nd	nd	nd	nd	nd	nd
C_{22}	nd	nd	nd	nd	nd	nd	nd	nd
C_{23}	–29.1 ± 0.5[a]	nd	nd	nd	–28.5 ± 0.4[a]	–29.4 ± 0.3	–29.4 ± 0.2	nd
C_{24}	nd	nd	nd	nd	nd	–30.4 ± 0.2[a]	nd	nd
C_{25}	nd	–29.1 ± 0.01	–29.2 ± 0.1	–29.3 ± 0.1	–29.0 ± 0.1	–29.3 ± 0.1	–29.5 ± 0.1	–29.6 ± 0.4[a]
C_{26}	nd	–31.0 ± 0.4[a]	nd	nd	–30.9 ± 0.3[a]	–31.8 ± 0.2	–31.6 ± 0.01	nd
C_{27}	–31.7 ± 0.4	–30.9 ± 0.1	–30.4 ± 0.2	–30.9 ± 0.3	–31.1 ± 0.02	–31.5 ± 0.1	–31.7 ± 0.05	–30.5 ± 0.2[a]
C_{28}	nd	–31.7 ± 0.1	–30.5 ± 0.1	–31.0 ± 0.4[a]	–32.0 ± 0.3	–32.8 ± 0.01	–32.8 ± 0.2	–30.5 ± 0.3[a]
C_{29}	–34.0 ± 0.5	–32.5 ± 0.4	–32.6 ± 0.1	–33.3 ± 0.1	–33.3 ± 0.005	–33.9 ± 0.1	–34.0 ± 0.1	–33.2 ± 0.3
C_{30}	–30.8 ± 0.03	–31.8 ± 0.2	–30.6 ± 0.1	–32.0 ± 0.3[a]	–32.0 ± 0.2	–34.0 ± 0.2	–33.5 ± 0.5	–30.3 ± 0.3
C_{31}	–33.2 ± 0.1	–32.4 ± 0.02	–31.7 ± 0.2	–32.3 ± 0.1	–32.2 ± 0.1	–33.4 ± 0.1	–33.0 ± 0.4	–31.3 ± 0.3
C_{32}	–31.7 ± 0.5	–30.7 ± 0.2	nd	–30.8 ± 0.5[a]	–31.3 ± 0.2	–33.6 ± 0.02	–32.5 ± 0.02	–30.7 ± 0.4[a]
C_{33}	–31.3 ± 0.02	–30.1 ± 0.4	–30.2 ± 0.1	–29.8 ± 0.002	–30.0 ± 0.2	–31.9 ± 0.1	–30.3 ± 0.4	–29.7 ± 0.1
C_{34}	–30.0 ± 0.1[a]	–30.0 ± 0.1	nd	–29.9 ± 0.4[a]	–29.8 ± 0.2	nd	nd	nd
C_{35}	–29.4 ± 0.1	–28.3 ± 0.5	–28.3 ± 0.1[a]	–28.0 ± 0.4[a]	–28.1 ± 0.2	–28.7 ± 0.2	–27.0 ± 0.4	–28.0 ± 0.1
C_{24}d	–33.1 ± 0.4	–32.8 ± 0.5	–32.9 ± 0.03	–32.8 ± 0.4	–32.8 ± 0.04	–32.6 ± 0.01	–32.4 ± 0.4	–33.0 ± 0.3
C_{30}d	–32.7 ± 0.2	–33.2 ± 0.5	–33.0 ± 0.05	nd	–33.1 ± 0.2	–33.5 ± 0.3	nd	–33.4 ± 0.05
Mean	–30.7	–30.8	–30.0	–30.5	–30.7	–31.7	–31.4	–30.4
Mean (C_{25}-C_{35})	–31.5	–30.8	–30.4	–30.7	–30.9	–32.1	–31.6	–30.4
Bulk	–23.2	–21.2	–20.9	–21.0	–22.2	–24.9	–22.4	–21.4

[a]Peaks between 200 and 500 mV, average of four injections. nd: not detected or not determined.

(*n*-alkanes typical from terrestrial plants) were found for the stations P2, P6, P7 and P8. Except for P2, these are the stations with the highest CPI values and more negative $\delta^{13}C$ for the TOC (Tables 3 and 6). This association suggests a predominance of biogenic *n*-alkanes from terrestrial origin that was already expected considering the influence of the mangrove forests located near to these stations. Similar results were obtained by Pearson and Eglinton,[13] who also found a ^{13}C-depletion for *n*-C_{29}, *n*-C_{31} and *n*-C_{33} probably associated with terrestrial C3 plant waxes. ^{13}C-Enriched values for the TOC (≥ –21.4‰) were found for stations P3, P4, P5 and P9, probably related to the intense input from the sewage discharges in the western portion of the bay.[41] This coincides with the results observed by Carreira *et al.*[29] in the same study area, where the stations located near the central area of the bay presented less negative $\delta^{13}C$ values.

For most samples the *n*-alkanes reached a strong minimum in $\delta^{13}C$ at *n*-C_{29}, becoming progressively ^{13}C-enriched up to *n*-C_{35}. This isotopic enrichment in compounds > *n*-C_{31} may be associated with oil contamination. Sun *et al.*[22] obtained $\delta^{13}C$ values between –26.8 and –24.9‰ for *n*-alkanes present in oils at different levels of biodegradation. These values are considerably more ^{13}C-enriched than those obtained for C3 plants.

Sediments receiving hydrocarbons from a mixture of sources usually present a "zigzag" pattern in the $\delta^{13}C$ signatures, where the odd-numbered *n*-alkanes are typically more depleted than adjacent even-numbered *n*-alkanes. This pattern occurs because of the high CPI values observed for the ^{13}C-depleted terrestrial plants in contrast with the low CPI values of the ^{13}C-enriched fossil fuels.[12,16,81] This pattern was not observed in this study and, according to Ahad *et al.*,[7] a less pronounced "zigzag" pattern associated with the ^{13}C-enrichment in longer-chain homologues could emphasize a greater contribution from petrogenic hydrocarbon.

Despite the minimum in *n*-C_{29}, no statistically significant differences were found in the $\delta^{13}C$ values between the odd

and even-chain *n*-alkanes (*t*-test: $t = 1.24$, $p = 0.24$, $\alpha = 0.05$). The average $\delta^{13}C$ was –30.9‰ for the odd-numbered *n*-alkanes (*n*-C_{25} to *n*-C_{35}) and –31.3‰ for the even-numbered *n*-alkanes (*n*-C_{26} to *n*-C_{34}), slightly more depleted than the results from Pearson and Eglinton[13] for Santa Monic Basin sediments showing averages of –30.0 and –29.9‰ for the odd and even-numbered *n*-alkanes (*n*-C_{24} to *n*-C_{33}), respectively. According to the authors, small isotopic differences between even and odd-numbered chains are typically associated with petroleum-derived products.

Station P7 has the most negative $\delta^{13}C$ values. This result, associated with the lower UCM concentration, indicates a smaller petrogenic influence inside the environmental protection area. As to the other stations, a large isotopic variation among the *n*-alkanes was not observed. According to Ishiwatari *et al.*,[16] this result would suggest a common source of the contaminant oil.

Conclusions

The UCM found in high concentrations was efficiently removed via $AgNO_3$-silica gel column combined with urea adduction for all sediment samples from Guanabara Bay without apparent losses of the compounds of interest, allowing further analysis of the compound-specific $\delta^{13}C$ of *n*-alkanes by GC-C-IRMS. Thus, the final conditions adjusted for the methods can be applied in future $\delta^{13}C$ analysis of *n*-alkanes in sediments from highly contaminated areas.

CPI and $\delta^{13}C$ results for the sediments suggest multiple sources and are within the same range of other contaminated environments. Stations P4 and P5, which showed higher concentrations for the LMW compounds, also had a more ^{13}C-enriched TOC and this could be an evidence of greater input of nutrients by the sewage discharges in this bay area and the consequent stimulation of primary production.

The most ^{13}C-depleted *n*-alkanes and TOC associated with higher CPI (> 4) for P6, P7 and P8, suggest a prevalence of biogenic *n*-alkanes from terrestrial origin for these stations in relation to others. This prevalence was greater in P7 station, located at northeast Guanabara Bay inside the environmental protection area, the less degraded region of the bay influenced by inputs from mangrove areas.

Small isotopic differences between the odd and even-numbered chains and enrichment in long-chain homologues indicate a significant petrogenic hydrocarbon contribution to the sediments of the bay. A possible explanation for the absence of a large isotopic variation in *n*-alkanes of most samples could be a common source of the oil which contaminates the bay and similar mixing rates between different terrestrial and autochthonous sources.

Results show that $\delta^{13}C$ of *n*-alkanes is a good tool that complement the identification of the sources and transformation of organic matter in a complex system as the Guanabara Bay. The next step for a better understanding of this system would be to include more data for the LMW homologues and develop a mixing model including the main sources and estimating the contribution by each of them.

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