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Multiple reaction monitoring tool applied in the geochemical investigation of a mysterious oil spill in northeast Brazil

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Abstract: In 2019, much of the northeastern coast of Brazil was impacted by a mysterious oil spill that caused an environmental disaster affecting 1009 beaches. Four samples were collected in the beaches between Sergipe and Pernambuco for geochemical characterization of the spilled oil and to compare with those main produced in Sergipe-Alagoas basin. Our approach in this evaluation was the use of a highly selective technique of sequential mass spectrometry by multiple reaction monitoring, to obtain the diagnostic ratios of hopanes and steranes biomarkers. Using these biomarkers ratios associated with multivariate statistical analysis, we found direct correlation between the spilled oil collected along the northeastern coast and no relationship between Sergipe-Alagoas basin crude oils was found. Furthermore, reported data for oils from Orinoco belt in Venezuelan basins were used for qualitative evaluation considering the indicative aspects suggested by the literature. Presence of highly specific biomarker 18α(H)-oleanane, and five other important diagnostic ratios evidenced correlation between the spilled oil and Naricual formation crude oils. Besides, due to the oleanane index, Ayacucho's crude oil presented the strongest factor of correlation with the spilled oil found on the northeast coast of Brazil.

Key words: Biomarkers, forensic geochemistry, oleanane, naricual formation, steranes, hopanes**.**

INTRODUCTION

One year after the environmental disaster caused by a massive spill of a mysterious petroleum material in the ocean, which reached the coast of Brazil, including the entire northeastern Brazilian coast, many questions remained unanswered. From the first appearance of large oil slicks on Brazilian beaches, on August 30, 2019, until March 19, 2020, 1009 Brazilian beaches (minimum distance of 1 km between them) were recorded as being affected by the oil (IBAMA 2020). This incident, which was the largest environmental disaster caused by oil spills in the country's history, caused damage to ecosystems, and socio-economic impacts in the areas affected (Araújo et al. 2020). Environmental studies and reports on the consequences for marine biodiversity and local populations made clear the extent of the damage, as well as a lack of information concerning the source of the oil (Magris & Giarrizzo 2020, Oliveira et al. 2019). According to the recent publication written by Zacharias et al. (2021), published investigations and studies indicate two hypotheses about the provenance of the oils spilled on the Brazilian coast, (a) an unidentified ship spilling crude oil into the ocean 700 km off the Brazilian coast, (b) a slow leakage of oil from an old or new shipwreck (Zacharias et al. 2021). Other authors also have released contributions that point to evidence of oil characteristics of Venezuelan oil fields (Lourenço et al. 2020, Oliveira et al. 2019).

Oil spills released into the environment are a global problem, so it is extremely important to be able to identify their sources. This can be achieved by characterization studies using petroleum biomarker compounds for correlation between oils. Comparison of the structures of these resistant compounds, such as those of the classes of terpanes and steranes, is essential for determining the source of the spill and the origin of the oil (Mulabagal et al. 2013, Shirneshan et al. 2016, Suneel et al. 2019). For this purpose, gas chromatography/quadrupole mass spectrometry (GC/qMS) has been an extremely effective tool, which is commonly used in two different acquisition methods, namely full scan (SCAN) and selected ion monitoring (SIM) aiming to perform the separation and identification of petroleum hydrocarbons (Borisov et al. 2019, Wang et al. 2006). Recently, gas chromatography/triple quadrupole mass spectrometry (GC-MS/MS) has been employed to develop more selective and sensitive methods for analyzing petroleum biomarkers, such as polycyclic aromatic hydrocarbons (PAHs), terpanes and steranes (Han et al. 2020, Qian et al. 2017).

GC-MS/MS operating in a Tandem mode promotes the selection of biomarker precursor ion in the first quadrupole (Q1) which pass to the collision cell (Q2) where they are fragmented by collision-induced dissociation (CID). Lastly, the third quadrupole (Q3) is set to allow to pass the biomarker structure-specific product ion. This approach is named multiple reaction monitoring (MRM) (Adhikari et al. 2017, Guan et al. 2010). In GC-MS/MS analysis, the use of MRM acquisition mode provides high selectivity, enabling accurate analysis of a large number of transitions with different *m/z* ratios. The MRM method eliminates problems related to interfering chromatographic peaks and improves the signal-to-noise ratio, compared to selective ion monitoring (SIM) mode, which is especially important in analyses of complex natural mixtures, such as crude oils under forensic investigation of oil spills (Borisov et al. 2019, Qian et al. 2017, Wang et al. 2006). Han et al. (2020) investigated the performance of the pseudo multiple reaction monitoring (PMRM), MRM, SIM, and SCAN acquisition modes for the quantification of PAHs present in oil samples, which revealed the superiority of the MRM method for this purpose (Han et al. 2020).

In a recent study by Brazilian researchers concerning the mysterious spill that reached the Brazilian coast in 2019, gas chromatography with flame ionization detection (GC-FID) and GC/MS were used to analyze 11 samples of petroleum material collected on different beaches in September 2019. The results for the identification of biomarkers and their diagnostic ratios indicated a pattern of similarity among 10 of these 11 samples (Lourenço et al. 2020). In other recent work, de Oliveira et al. (2020) used geochemical analytical techniques to characterize oil spill samples collected in the Northeast region of Brazil, comparing oil samples from the environmental disaster and from national and foreign basins, aiming to identify similarities among the samples.

Despite disclosure of the likely Venezuelan origin of the petroleum material from this spill, many questions still remain concerning this disaster. There is a lack of specific information about the original oil, such as the location of the producing basin from which this oil was initially extracted. Furthermore, doubts remain regarding the similarities of the oleaginous contents of the dense and viscous materials found at the 1009 locations in the eleven Brazilian states affected, and whether they were actually from the same spill. So far, nothing is known about the possibility that other incidents could have occurred at the same time, but have not yet been discovered or reported, which could have contributed petroleum material additional to that associated with the disaster. Furthermore, comparison of the chromatographic profiles of the oil samples and oils from Venezuelan basins could be performed using published analytical data for constituents of Venezuelan oils (Hinkle et al. 2008, López & Mónaco 2017).

Knowing that oil spills are common and often inevitable in industrial processes, it is necessary to assess the possible occurrence of simultaneous spills during the same period, starting on August 30, 2019. Due to the proximity of the Sergipe-Alagoas basin to the coasts of the states of Bahia, Alagoas, Pernambuco, and Sergipe, described as being most affected in terms of the volumes of oil reaching the beaches (Oliveira et al. 2019), it has been suggested that oils from this basin could have contributed to the disaster. Therefore, the aims of this work were to uses the recent and highly selective technique of Gas Chromatography/Mass Spectrometry by Multiple Reaction Monitoring (GC-MS/MS-MRM) to obtain hopanes and steranes diagnostic ratios of spilled oil samples collected in the states of Sergipe and Pernambuco, as well as also to compare with those obtained to crude oils from the Sergipe-Alagoas basin for forensic purposes of comparison and within complementary approach for the geochemical characteristic analysis of the spilled oils with those reported in the literature for oils produced in the Venezuelan basins.

MATERIALS AND METHODS

Oil spill sample collection

In August 2019, large amounts of crude oil, spread by marine currents, were detected along the northeastern coastline of Brazil, with the spill subsequently reaching the entire northeastern and part of the southeastern coastline. No oil companies operating in Brazilian waters reported any oil spill incidents. The amount of petroleum released, its source, and when, where, and how the spill occurred still remain unknown (Lourenço et al. 2020). In the present work, four samples of crude oil related to the spill event were collected, including two from the Sergipe coast and two from the Pernambuco coast. The highly viscous liquid samples (see Supplementary Material - Figure S1) were collected on the beach mixed with sand, without any sediments near the area. They were collected with the assistance of a metal spatula on the surface of the oil slicks, and stored in clean glass bottles for further analysis.

Five crude oil samples from the main currents exploited in Sergipe-Alagoas basin were supplied by an oil company operating in this basin. No information was provided concerning the collection location and time, because this information was confidential. Details concerning sample location, code, and acquisition date are provided in Table I and collection points are shown in Figure 1.

Sample preparation

Spilled oil samples arrived at the laboratory in a 40 mL glass vial with PTFE septum. 2 g of the mixtures were extracted five times with 5 mL of dichloromethane (Merck, purity ≥ 99.5%) (John et al. 2014, Shirneshan et al. 2016). At each extraction, the sample was centrifuged, and the organic phase was relocated to a flat bottom flask, which had its mass previously measured. In the fourth extraction, the solvent was already colorless. Then, the dichloromethane was removed at a rotary evaporator, leaving a brown oily residue which had their mass determined. 1 mg of the residue was re-dissolved in 1 mL of dichloromethane for GC-MS/MS analysis. Crude oil samples provided by the oil company were directly dissolved in 1 mL of dichloromethane, yielding solutions of 1 mg mL^{-1}. To perform quality control, the absolute area of 17β,21(H) β-hopane (H30) compound from the four extracted oil samples were computed and their relative standard deviation (RSD) was performed, resulting in a value of 10% of RSD.

Instrumentation and GC-MS/MS conditions

For determination of the *n*-alkanes profiles, the crude oil solutions were analyzed by gas chromatography/triple quadrupole mass spectrometry (GCMS-TQS 9000, Thermo Scientific), in electron ionization (EI) mode. Chromatographic separation employed an NA-5MS capillary column (5% phenyl, 95% dimethylpolysiloxane). The sample injection volume was 1 µL, in split mode (1:30), and the injector temperature was 290 °C. The oven temperature was programmed from 60 to 310 °C, at 2 °C min^{-1}. Helium (99.995% purity) was used as the carrier gas, at a constant flow rate of 1.0 mL min^{-1}. The mass spectrometer ion source temperature and the interface temperature were both set at 300 °C. The total chromatographic run time was 125 min.

Gas chromatography with flame ionization detection (GC-FID) was used for the determination of pristane and phytane (Figure S2), employing a LECO/Agilent 7890A instrument fitted with a Zebron ZB-5 capillary column (30 m x 0.32 mm, 0.25 µm, Phenomenex, Macclesfield, UK). The column stationary phase was 5% phenyl/95% dimethylpolysiloxane and the carrier gas was hydrogen, at a constant flow rate of 1 mL min^{-1}. The oven temperature program was 70 °C (maintained for 1 min) with ramps of 20 °C min^{-1} to 170 °C and then with heating rate of 2 °C min^{-1} to 300 °C and held for 10 min. The total chromatographic run time was 81 min and chromatograms are available in the Figure S3.

The biomarkers were determined using a gas chromatography/triple quadrupole mass spectrometry system (GCMS-TQ8040, Shimadzu) operated in electron ionization (EI) mode. Chromatographic separation was performed with an SH-RTX5SilMS capillary column (Crossbond 1,4-bis(dimethylsiloxy)phenylene dimethylpolysiloxane, 30 m x 0.25 mm i.d 0.25 µm film thickness, Restek, USA). The sample injection volume was 1 µL, in splitless mode, and the injector temperature was 300 °C. The oven temperature was programmed from 70 to 325 °C, at 3 °C min^{-1}. Helium (99.995% purity) was used as the carrier gas, at a constant flow rate of 1.0 mL min^{-1}. The mass spectrometer ion source temperature was 280 °C and the interface temperature was 290 °C. The collision gas was argon (99% purity) and the collision energy was 12 eV. The total chromatographic run time was 85 min.

Based on the MRM method created by Mei et al. (2018) and Prata et al. (2016), the extracted ion chromatographic peaks of the target analytes were monitored using absolute retention times. Information concerning compound names, codes, transitions, and retention times is summarized in Table II.

Statistical analysis: chemometric methods

The GC-MS/MS data obtained in MRM mode were treated to obtain the diagnostic ratios. The values were first normalized (using Excel 360) to reduce data variability unrelated to the chemical composition, followed by application of multivariate data analysis, using OriginPRO for Windows (trial version) software. Finally, several chemometric techniques were applied for assessment of correlations among the oil spill samples and suspected source oils.

Table I. Sergipe-Alagoas basin crude oils and samples of weathered crude oil collected from sites along the Sergipe and Pernambuco coasts between September 28, 2019, and October 26, 2019.

Name	Code	Physical state	Location	Sampling date
Sample G	1	Weathered	8°23’16.0”S, 34°57’55.4”W	10/20/2019
Sample P	2	Weathered	8°28’11.0”S, 34°59’30.5”W	10/10/2019
Sample 3	3	Weathered	10°52’49.4”S, 36°58’39.1”W	09/28/2019
SEMA-CM	4	Weathered	10°48’44.2”S, 36°54’80.7”W	10/26/2019
Piranema	A	Crude	Not provided	Not provided
Camorim	B	Crude	Not provided	Not provided
Alagoano	C	Crude	Not provided	Not provided
Sergipe-Terra	D	Crude	Not provided	Not provided
Atalaia-Terra	E	Crude	Not provided	Not provided

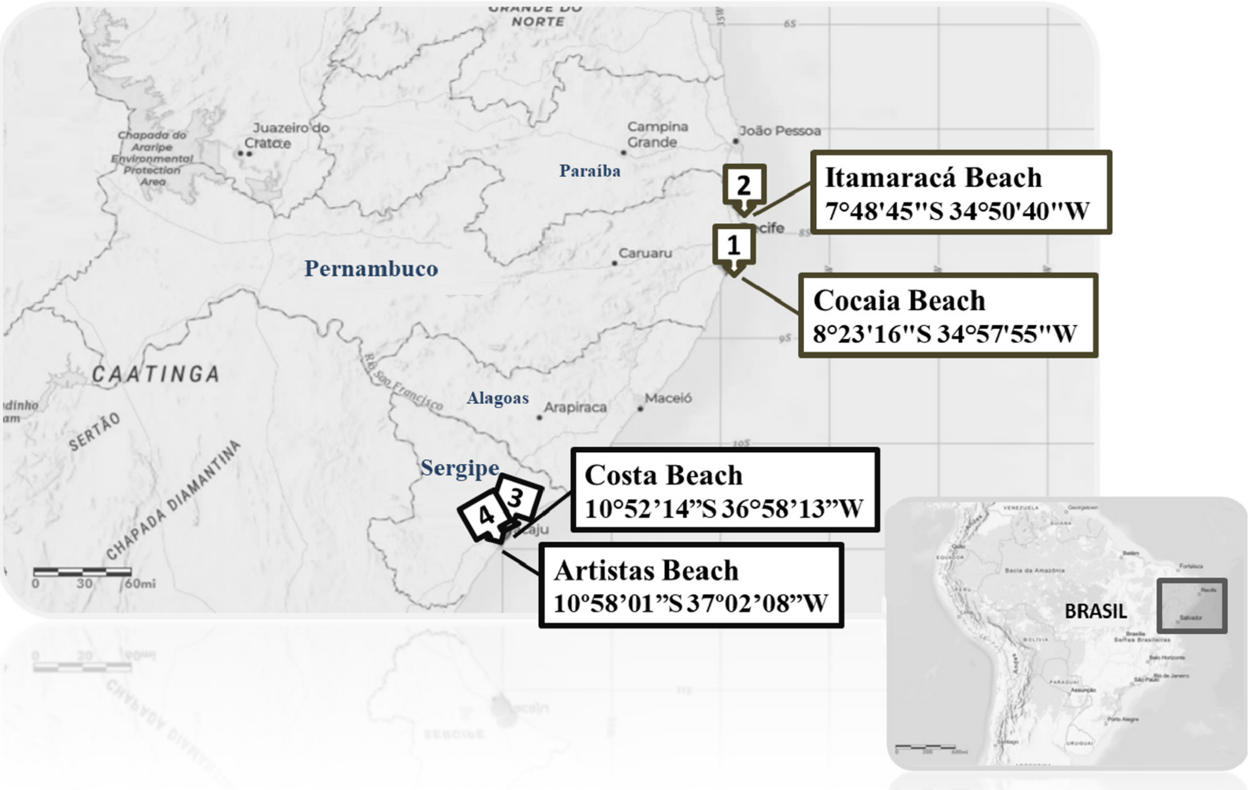

Figure 1. Map showing the oil sample collection locations on the coasts of the states of Sergipe and Pernambuco.

Table II. **Transitions (precursor ions and product ions) used for the investigation and identification of steranes and hopanes in MRM mode.**

Compound name	Code	Molecular formula	Transition *(m/z)*	Retention time *(min)*
5α(H),14α(H),17α(H)-cholestane (20R)	C27R	$C_{27}H_{48}$	372>217	62.486
5α(H),14ß(H),17ß(H)-cholestane (20S)	C27bbS	$C_{27}H_{48}$	372>217	62.069
5α(H),14ß(H),17ß(H)-cholestane (20R)	C27bbR	$C_{27}H_{48}$	372>217	61.813
5α(H),14α(H),17α(H)-cholestane (20S)	C27S	$C_{27}H_{48}$	372>217	61.631
5α(H),14α(H),17α(H)-ergostane (20R)	C28R	$C_{28}H_{50}$	386>217	64.834
13β(H),17α(H)-diacholestane (20R)	DIA27R	$C_{27}H_{48}$	372>217	58.816
13β(H),17α(H)-diacholestane (20S)	DIA27S	$C_{27}H_{48}$	372>217	58.058
5α(H),14ß(H),17ß(H)-ergostane (20S)	C28S	$C_{28}H_{50}$	386>217	64.319
5α(H),14ß(H),17ß(H)-ergostane (20R)	C28bbR	$C_{28}H_{50}$	386>217	64.057
5α(H),14α(H),17α(H)-ergostane (20S)	C28bbS	$C_{28}H_{50}$	386>217	63.868
5α(H),14α(H),17α(H)-stigmastane (20R)	C29R	$C_{29}H_{52}$	400>217	66.752
5α(H),14ß(H),17ß(H)-stigmastane (20S)	C29bbS	$C_{29}H_{52}$	400>217	66.099
5α(H),14β(H),17β(H)-stigmastane (20R)	C29bbR	$C_{29}H_{52}$	400>217	65.892
5α(H),14α(H),17α(H)-stigmastane (20S)	C29S	$C_{29}H_{52}$	400>217	65.564
5α(H),14α(H),17α(H)-24-iso-propylcholestane (20R)	ISO30R	$C_{30}H_{54}$	414>217	68.507
5α(H),14ß(H),17ß(H)-24-iso-propylcholestane (20S)	ISO30bbS	$C_{30}H_{54}$	414>217	68.021
5α(H),14ß(H),17ß(H)-24-iso-propylcholestane (20R)	ISO30bbR	$C_{30}H_{54}$	414>217	67.779
5α(H),14α(H),17α(H)-24-iso-propylcholestane (20S)	ISO30S	$C_{30}H_{54}$	414>217	67.243
13β(H),17α(H)-dia-24-iso-propylcholestane (20R)	DIA30R	$C_{30}H_{54}$	414>217	63.383
13β(H),17α(H)-dia-24-iso-propylcholestane (20S)	DIA30S	$C_{30}H_{54}$	414>217	62.528
18α(H)-22,29,30-trisnorhopane (Ts)	Ts	$C_{27}H_{46}$	370>191	63.038
17α(H)-22,29,30-trisnorhopane (Tm)	Tm	$C_{27}H_{46}$	370>191	63.986
17α(H),21ß(H)-30-norhopane (C29Hop)	H29	$C_{29}H_{50}$	398>191	66.882
C3017α(H)-diahopane	C30Diahop	$C_{30}H_{52}$	412>191	67.352
17α(H),21ß(H)-hopane (C30Hop)	H30	$C_{30}H_{52}$	412>191	68.659
17β(H),21α(H)-hopane (C30baHop)	MOR30	$C_{30}H_{52}$	412>191	69.514
18α(H)-oleanane + 18β(H)-oleanane	OL	$C_{30}H_{52}$	412>191	68.346
17α(H),21ß(H)-homohopane (22S)	H31S	$C_{31}H_{54}$	426>191	70.736
17α(H),21ß(H)-homohopane (22R)	H31R	$C_{31}H_{54}$	426>191	70.978
Gammacerane	GAM	$C_{31}H_{54}$	412>191	71.356
17α(H),21ß(H)-bishomohopane (22S)	H32S	$C_{32}H_{56}$	440>191	72.379
17α(H),21ß(H)-bishomohopane (22R)	H32R	$C_{32}H_{56}$	440>191	72.706

RESULTS AND DISCUSSION

Fingerprint of *n*-alkanes from the spilled oil samples were performed using the reconstructed GC-MS/MS ion chromatograms (RICs) of the *m/z* 85 ion. A geochemical approach employing a series of biomarker ratios was then used to investigate possible correlations between the oil spilled samples and the five crude oil samples supplied by the oil company. The results were subsequently submitted to multivariate statistical analysis. Two possibilities were considered: (i) at least one of the crude oil samples supplied would be related to the spill, and (ii) no sample, among those supplied, would be related to the spill. The data for the spilled crude oil were also compared to the data available for crude oils from Venezuelan basins, which were suspected sources of the spill.

GC-MS/MS-RIC chromatographic profiles of *n*-alkanes in the oil spill samples

Oil fingerprinting is one of the key approaches for distinguishing and differentiating the sources of unknown oil and associated refined products released into the environment (Bayona et al. 2015). Normally, analysis by GC-FID is used in the first stage of investigation of oil-oil and oil-source rock correlations. However, GC-MS/MS total ion current chromatograms (TICCs) are similar and can provide the same information regarding the sample profiles. Furthermore, in the absence of direct full scan determination of *n*-alkanes, reconstructed ion chromatograms (RICs) can be used to obtain the *n*-alkanes profiles (Brodskii et al. 2010).

In order to increase sensitivity towards these compounds and improve visualization of the *n*-alkanes profiles, analysis of the paraffins distributions was performed by GC-MS/MS-RIC, using the *m/z* 85 ion (Figure 2) (Mei et al. 2018). Samples 1, 3, and 4, presented the same paraffinic compounds pattern, ranging from *n*-C_{12-13} to *n*-C_{33-34}, with a unimodal distribution skewed towards medium- and long-chain *n*-alkanes, characteristic of terrigenous organic matter inputs (Killops & Killops 2005, Tissot & Welte 1985). However, sample 2, which had been collected later on the Pernambuco coast (in October 2019), did not show the same *n*-alkanes range. The depletion of paraffinic compounds could be attributed to weathering processes that are known to affect oils released into the environment, which in this case was mainly attributed to the evaporation process (Stout & Wang 2007, Ward & Overton 2020).

According to the biodegradation index proposed by K.E. Peters et al. 2005b, due to the presence of *n*-alkanes series, it is suggested that these oils were not biodegraded. However, 25-norhopanes were detected by GC-MS/MS-SIM monitoring ion *m/z* 177 for spilled oil samples (Figure S4), indicating some degree of biodegradation (Bennett et al. 2006). It is not appropriate to say that these degree of biodegradation is resulting of the contact of the oils with the environment, due to the type of marine environment and their unknown time of exposure (Lima et al. 2021). Nonetheless, their coexistence in the same weathered oil sample may result from the mixing of oils from, at least, two separate oil during the reservoir filling process (Li et al. 2018).

The profiles of the Sergipe-Alagoas basin crude oils (Figure 2) showed the presence of light hydrocarbons, with high intensities for *n*-alkanes. The paraffinic compounds ranged from *n*-C_7 to *n*-C_{37}. Most of the profiles were similar, with bimodal patterns skewed towards short-chain *n*-alkanes, with the exception of oil A, for which the distribution presented a unimodal pattern skewed towards short-chain *n*-alkanes and these fingerprints can indicate inputs of certain types of source organic matter.

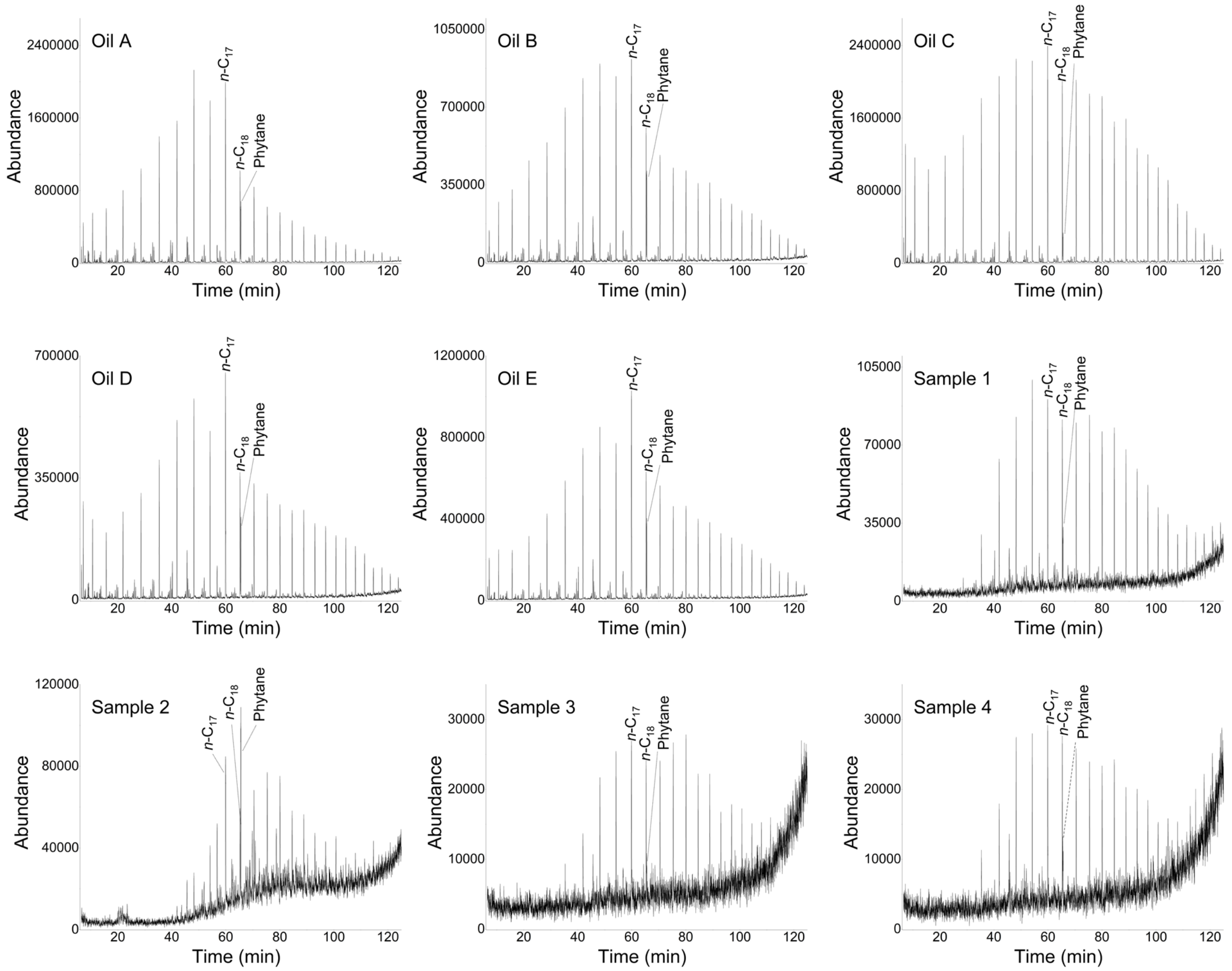

Figure 2. Reconstructed *m/z* 85 ion chromatograms for evaluation of n-alkanes distribution patterns.

Bimodal *n*-alkane distributions and those skewed towards the range from *n*-C_{23} to *n*-C_{31} are usually associated with terrigenous higher plant waxes, as observed for oils B, C, D, and E. For oil C, the distribution indicated both terrigenous and marine contributions, with the input from algal organic matter having a higher contribution (Peters et al. 2005a, Tissot & Welte 1985).

Comparison of the *n*-alkanes profiles for the spilled oils with those for Venezuelan crude oils and source rocks, reported by López & Mónaco (2017), showed that only two crude oils (Los Manueles and Ayacucho) presented profiles similar to those of the spilled oils. For the source rocks, the most similar *n*-alkanes profiles were those of the Naricual and San Antonio formations. Among these Venezuelan sources, the Ayacucho and Naricual *n*-alkanes distribution patterns showed high similarity to those of the spilled oils. However, as we do not know for how long these oils have been spilled into the environment, any assumptions regarding correlation of *n*-alkanes profile will present risk of bias or miss correlation, mainly because these compounds are too susceptible to weathering processes (Islam et al. 2013).

Biomarkers: organic matter type and sedimentation environment

The organic matter inputs and depositional conditions of the sediments that become source rocks are primary determinants of the biomarker fingerprints of source rock extracts and crude oils. Under certain depositional conditions, large populations of one or several organisms can produce abundant supplies of one or more precursors that can give rise to diagnostic biomarkers (Peters et al. 2005a). Therefore, diagnostic ratios were obtained using the peak areas of the organic matter and depositional environment biomarkers identified in the samples. Comparisons were made among the results obtained for the spill oil samples and the Sergipe-Alagoas basin crude oils. The spill oils were also compared, using similarity correlations, with data available in the literature for oil from Venezuelan basins.

For the saturated hydrocarbon fractions of rock extract and petroleum, oleanane is typically analyzed by GC/MS, with selected ion monitoring (SIM) of *m/z* 191 and other fragments. One of the disadvantages of using this operating mode is that other compounds with similar retention times and insufficiently distinguishable mass spectra occur in small amounts and can interfere in the GC/MS-SIM analysis (Moldowan et al. 1994). However, when MRM operating mode is used, it can improve the analysis of this biomarker (Moldowan et al. 1994, Qian et al. 2017). Oleanane present in crude oils and rock extracts has been used as a biomarker for both source input and geological age (Matapour & Karlsen 2018). This biomarker, which has two isomers, 18α(H)-oleanane and 18β(H)-oleanane, is only found in Tertiary and Cretaceous (<130 million years) rocks and oils, and is highly specific for angiosperm plant inputs (Wang et al. 2006). Since it is well known as a specific biomarker, along with gammacerane and β-carotane, its use as geochemical forensic approachesis fundamental (Boehm et al. 1997, Quintero et al. 2012, Stout & Wang 2016).

All the crude oil spill samples showed the presence of oleanane (18α(H)-oleanane + 18β(H)-oleanane) (Figure 3), but it was not observed in the crude oils from the Sergipe-Alagoas basin. The oleanane index (OI% = OI/(OI+H30)) values (Table III) indicated limited higher plant inputs (from angiosperms) during organic matter deposition, together with variations in terrigenous organic matter inputs during deposition of the source rocks of these oils (Peters et al. 2005b). The oleanane index values for the spill oil samples were in the range 6-16%, with the lowest value for sample 1 (OI% = 6). These results were in good agreement with the values in the range 8-14% reported by Lourenço et al. (2020). The presence of this biomarker was observed by de Oliveira et al. (2020), although they did not take into account the area of 18α(H)-oleanane, adjacent to 17α(H),21ß(H)-hopane in the *m/z* 191 chromatogram.

Since hopanes and oleananes are among the last saturated molecules to be biodegraded in oil spills, the distributions of these compounds can act as fingerprints for identifying oils that originate from the same source (Stout & Wang 2016). The presence of oleanane has been reported in the Venezuelan crude oils La Victoria, Guafita, Caipe, Silvestre, Sinco, Silván, Palmita, Hato, Mingo, Maporal, Junín, Ayacucho, Carabobo, and Los Manueles. The San Antonio and Naricual formations are the only Venezuelan source rocks with oleanane in their compositions. The OI% values reported for the Venezuelan oils are 14-16% (La Victoria and Guafita), 9-15% (Caipe, Silvestre, Sinco, Silván, Palmita, Hato, Mingo, and Mapora), 3-10% (Júnin), 5-11% (Ayacucho), 8% (Carabobo), and 11% (Los Manueles). The OI% values for the source rocks are 3-16% (San Antonio) and 23-24% (Naricual). The greatest similarity with the oleanane index values obtained here for the spill samples was observed for the Orinoco heavy oil belt oils (Júnin, Ayacucho, and Carabobo), Los Manueles,

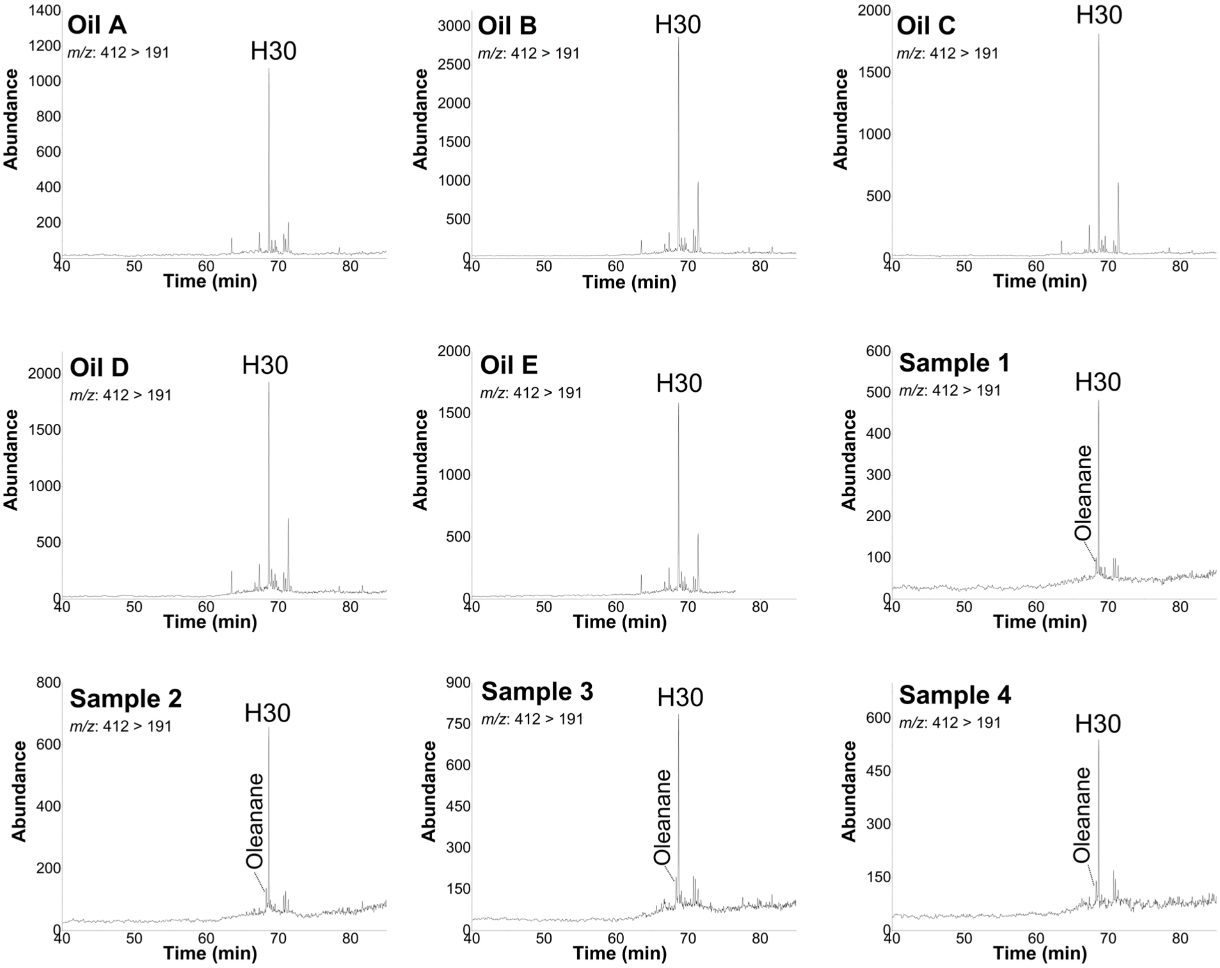

Figure 3. Expansion of the chromatograms for the transition *m/z* 412>191 used to identify the presence or absence of the 18α(H)-oleanane biomarker in the samples.

Caipe, Silvestre, Sinco, Silván, Palmita, Hato, Mingo, and Maporal.

The gammacerane index values (GI% = GAM/(GAM+H30)) can reflect variations of stratification conditions in the water column. Gammacerane was detected in all the samples (Sergipe-Alagoas basin and spill crude oils), with the lowest GI% value obtained for sample 4 (3%) and the highest for oil D (23%). The gammacerane index values for the spill oils ranged from 3 to 8, characterizing marine calcareous, siliciclastic, or deltaic source rock. On the other hand, the oils from the Sergipe-Alagoas basin showed values in the range 12-23%, characteristic of marine carbonate source rocks (Bost et al. 2001, Peters et al. 2005b, Peters & Moldowan 1993). Among the Venezuelan crude oils in which oleanane was detected, gammacerane was found in La Victoria, Guafita, Los Manueles, Caipe, Silvestre, Sinco, Silván, Palmita, Hato, Mingo, Maporal, Junín, Ayacucho, and Carabobo (López & Mónaco 2017), with the lowest GI% values for Junín and Ayacucho. However, the values were not close to those observed for the spill oils.

Considering the organic matter type, three groups of sterenes, which are subsequently converted into steranes, appear differently in the biosphere, besides having slightly different biosynthesis pathways. For example, cholesterol, which is transformed into cholestane (C_{27}, identified from the transition *m/z* 372>217), is widely distributed and occurs in nearly all organisms, including plants and animals. Stigmasterol, subsequently converted into stigmastane (C_{29}, identified from the transition *m/z* 400>217), is predominantly found in terrestrial higher plants. Ergosterol, subsequently transformed into ergostane (C_{28}, identified from the transition *m/z* 386>217), is a typical component of fungi, while brassicasterol is found in several unicellular algae (Killops & Killops 2014, Schwarzbauer & Jovančićević 2016).

Evaluation of the ternary-type ratios for the C_{27}-C_{28}-C_{29} regular sterane biomarkers (Table III) showed that the majority of the crude oil samples analyzed were predominantly associated with terrigenous organic matter inputs, since the C_{29} regular sterane was relatively more abundant than the C_{27} and C_{28} steranes (Peters et al. 2005a). The exceptions were oil C and samples 2, where C_{27} was the major regular sterane, characteristic of predominantly marine organic matter input. In comparison with the Venezuelan oils, all the samples had a greater relative abundance of C_{27}, compared to the other regular steranes, suggesting that all the samples (not just oil C and samples 2) had predominantly marine organic matter inputs (Escobar et al. 2012, López & Mónaco 2017).

These ratios were calculated using the sum of the relative abundances of steranes identified in all transitions for *m/z* >217 over the sum of the relative abundances of hopanes identified in all transitions for *m/z* >191 (C_{27-29} ααα [20S+20R] and αββ [20S+20R] steranes/ C_{29-33} αβ hopanes [22S+22R]) (Adegoke et al. 2015). All the crude oils showed values higher than 1, ranging from 1.16 (sample 2) to 3.54 (oil B), indicative of inputs of marine organic matter, with major contributions from planktonic and/or benthic algae. Low steranes/hopanes ratios suggest a greater influence of terrigenous and/or microbially reworked organic matter. Therefore, the values near 1 found for samples 1, 2, 3, and 4, as well as oil C (1.16–1.61), indicated greater contributions of terrigenous organic matter, with slight inputs of marine organic matter (Peters et al. 2005a). In comparison to the data for Venezuelan crude oils reported by Bost et al. (2001), the values of this ratio for the spilled oils were similar to those observed for the Easter basin, specifically the Maturin-Temblador sub-basin (steranes/terpanes = 1.2) (Bost et al. 2001, López & Mónaco 2017).

The complementary biomarker ratio for the depositional environment, C_{31}22R homohopane/C_{30} hopane (H31R/H30), is useful for discrimination between marine and lacustrine source rock depositional environments. This ratio is obtained using the transitions *m/z* 412>191 and *m/z* 416>191. Unlike crude oils from lacustrine source rocks, oils from marine shale, carbonate, and marl source rocks generally show high values (H31R/H30 >0.25). The values obtained for this ratio (Table III) ranged from 0.12 to 0.37, with all the spill oils presenting values higher than 0.25. Therefore, in contrast to the Sergipe-Alagoas basin crude oils, those from the oil spill originated from marine carbonate, shale, or marl source rock (Killops & Killops 2014, Peters et al. 2005b). With this information, together with the steranes/hopanes ratios and Pr/Ph >1, it could be inferred that the spill samples were from marine shale sedimented under suboxic conditions. Correlating the H31R/H30 values to those available for Venezuelan crude oils, and also considering the presence of oleanane, similarity was obtained with oils from the Eastern Basin (H31R/H30 = 0.23-0.32, La Victoria, Guafita, Caipe, Silvestre, Sinco, Silván, Palmita, Hato, Mingo, and Maporal) and the Naricual formation source rock (H31R/H30 = 0.21-0.28) (López & Mónaco 2017).

Thermal evolution

Thermal maturity, or thermal evolution, refers to the extent of the heat involved in the evolutionary thermodynamic processes of converting organic matter into oil (Peters et al.

Table III. Oil sample biomarker ratios for identification of organic matter sources and depositional environments.

Biomarker ratios	Sample 1	Sample 2	Sample 3	Sample 4	Oil A	Oil B	Oil C	Oil D	Oil E
Ts/Tm[a]	0.58	0.77	0.90	0.64	0.83	0.91	1.12	0.91	0.99
Ts/(Ts+Tm)[a]	0.37	0.43	0.47	0.39	0.45	0.48	0.53	0.48	0.50
MOR30/H30[a]	0.07	0.05	0.03	0.04	0.06	0.06	0.06	0.06	0.08
H32S/H32R+H32S[a]	0.60	0.55	0.67	0.70	0.59	0.58	0.61	0.56	0.59
C29S/(C29S+C29R)[a]	0.53	0.50	0.28	0.55	0.42	0.49	0.28	0.38	0.37
C29bb(S+R)/ (C29bb(S+R)+C29aa(S+R))[a]	0.48	0.55	0.61	0.54	0.49	0.42	0.31	0.37	0.37
%$C_{27\,=}$ C27 20R/((C27–C29)20R	43%	47%	42%	38%	37%	34%	56%	36%	35%
%$C_{28\,=}$ C28 20R/((C27–C29)20R	11%	7%	9%	21%	21%	22%	20%	21%	21%
%C_{29} = C29 20R/((C27–C29)20R	46%	46%	48%	41%	42%	44%	23%	43%	44%
Pr/Ph[b]	1.69	1.73	1.65	1.88	0.81	0.89	1.46	0.92	0.94
H31R/H30[b]	0.27	0.37	0.26	0.27	0.18	0.20	0.12	0.18	0.17
GI%[b] = GAM/(GAM+H30)	8%	7%	3%	1%	12%	21%	17%	23%	19%
OI%[b] = OL/(OL+H30)	6%	15%	13%	16%	0%	0%	0%	0%	0%
Steranes/Terpanes[b] [*]	1.19	1.16	1.26	1.60	2.81	3.54	1.61	3.02	2.85
C27 bb(R+S)/C29bb(R+S)[c]	1.06	0.83	0.95	0.74	0.61	0.55	0.69	0.42	0.43
C27S/C27R[c]	1.02	1.04	0.97	1.05	0.88	1.09	0.53	0.83	0.88
C28S/C28R[c]	4.63	6.41	5.85	2.31	0.58	0.65	0.03	0.31	0.31
C29S/C29S[c]	1.13	1.00	0.38	1.24	0.72	0.94	0.40	0.61	0.59
DIA27/C27[c]	0.61	0.48	0.74	0.67	3.05	0.54	0.13	0.64	0.63
ISO30S/ISO30S+ISO30R[c]	0.51	0.58	0.42	0.48	0.28	0.44	0.10	0.43	0.43
ISO30bb(S+R)/ ISO30bb(S+R)+ISO30(S+R)[c]	0.63	0.40	0.55	0.29	0.41	0.46	0.18	0.29	0.33
DIA30S/(DIA30S+DIA30R)[c]	0.39	0.11	0.55	0.42	0.06	0.25	0.00	0.06	0.24
H29/H30[c]	0.51	0.50	0.53	0.41	0.23	0.29	0.21	0.30	0.32
GAM/H30[c]	0.09	0.07	0.03	0.01	0.13	0.27	0.21	0.30	0.23
H31S/H31R[c]	1.57	1.12	1.61	1.37	1.65	1.43	1.28	1.36	1.52
H32S/H32R[c]	1.53	1.22	2.03	2.39	1.43	1.37	1.58	1.25	1.41
C30Diahop/H30[c]	0.01	0.04	0.04	0.02	0.09	0.08	0.11	0.12	0.10

[a] Biomarker ratio used for thermal evolution.

[b] Biomarker ratio used for depositional environment and organic matter input.

[c] Biomarker ratio used only for oil-oil correlations.

* (C27–29 ααα [20S+20R] and αββ [20S+20R] steranes/ C29–33 αβ hopanes [22S+22R]).

2005b). Calculations of the diagnostic ratios were performed using the areas of thermal maturity biomarkers identified in the samples, considering the results obtained for the spill oils and the Sergipe-Alagoas basin crude oils. Comparison was also performed using similarity correlations for the spill oils and the literature data for Venezuelan oil containing the oleanane biomarker as one of its constituents.

The ratio between the relative areas of terpane biomarkers 18α(H)-22,29,30-trisnorneohopane (Ts) and 17α(H)-22,29,30-trisnorhopane (Tm), identified from the transition *m/z* 370>191, is used along with other ratios to obtain information regarding thermal evolution, which depends on temperature and thermal stress. Since the compound Ts is thermally more stable, relative to Tm, the concentrations of Ts in thermally evolved oils are higher, compared to Tm. The presence of significant concentrations of Tm in oils generates low values for the Ts/Tm ratio, which varies from 0 to 1, as well as values less than 1 for the Ts/(Ts+Tm) ratio, which may indicate carbonate source rocks (Peters et al. 2005a, b). The four oil samples collected on the beaches showed similar Ts/(Ts+Tm) ratio values between 0.37 and 0.47, with a higher concentration of the Tm biomarker, which is less thermally stable than Ts. The Ts/Tm ratio values were between 0.58 and 0.90. The samples of oil produced in the Sergipe-Alagoas basin presented values between 0.48 and 0.53 for the Ts/(Ts+Tm) ratio and between 0.83 and 1.12 for the Ts/Tm ratio. The values indicated that oils B, C, D, and E were from rocks of greater thermal maturity, compared to the others. In comparison with oils from Venezuelan basins, the Ts/(Ts+Tm) ratio values for the spill oils collected on the beaches (0.37-0.47) were similar to those available in the literature for the La Victoria and Guafita oils (0.38-0.50), and the Caipe, Silvestre, Sinco, Silván, Palmita, Hato, Mingo, and Maporal oils (0.34-0.50). The values obtained were also similar to those for the source rocks. The Ts/(Ts+Tm) values for samples 1, 2, 3, 4, and 5 (0.37-0.47) were all within or very close to the range described for the San Antonio source rock (0.35-0.53), while the values for samples 1 (0.37) and 4 (0.39) were within the range reported for the Naricual source rock (0.30-0.40) (López & Mónaco 2017).

The isomerization of C32-homohopanes R and S, which are terpane biomarkers identified by GC/MS/MS using the transition *m/z* 440>191, is also related to thermal maturity. For these biomarkers, the R isomer converts to the more stable S isomer with increase of the oil formation temperature. Hence, the S isomer has greater abundance in more thermally evolved oils (Waples & Machihara 1991). During maturation, the H32S/H32R+H32S ratio values increase from 0, when there is only the R isomer, to approximately 0.6 (0.57-0.62), when a balance is reached between the concentrations of the isomers present, indicating maturity equilibrium (Peters et al. 2005b). For the oil samples collected on the beaches of the states of Pernambuco and Sergipe, the values of this ratio for samples 1, 2, 3, and 4 were between 0.55 and 0.70, very close to the maturity equilibrium range, indicating that these were thermally mature oils. In the case of the oil samples from the Sergipe-Alagoas basin, the values found for oils A, B, C, D, and E were between 0.56 and 0.61, all within or very close to the maturity equilibrium range. Correlating the values obtained for this diagnostic ratio to the literature values reported for Venezuelan oils, it could be seen that the value for sample 2 (0.55) was within the range for the Venezuelan basins of La Victoria and Guafita (0.53-0.60). On the other hand, samples 1, 3, and 4 showed similar values, but above those reported for other Venezuelan basins by López & Mónaco (2017). Considering the source rocks, only sample 2 showed a value of this diagnostic ratio (0.55) very close to the range of values reported for the San Antonio rock (0.56-0.62).

Since moretanes are less thermally stable than hopanes, the concentration of the biomarker C_{30}17β(H),21α(H)-moretane (Mor) tends to decrease, relative to the concentration of the biomarker C_{30}17α(H),21β(H)-hopane, with increasing maturity (Peters et al. 2005b, Waples & Machihara 1991). As maturity increases, the Mor/Hop ratio decreases, with values of around 0.8 found for immature rocks, while values for mature rocks may be less than 0.15 and as low as 0.05 (Peters et al. 2005b). The Mor30/H30 (Mor/Hop) biomarker ratios obtained for the four samples collected on the beaches, using the transition *m/z* 412>191, were between 0.03 and 0.07. The Mor/Hop ratios for samples 2 and 1 (0.5 and 0.7, respectively) were within the range of values found for oils from mature generator rocks (>0.15-0.05). Samples 3 and 4 showed values of 0.03 and 0.04, respectively, suggesting that these oils had greater thermal maturity, compared to the others. For the Sergipe-Alagoas basin crude oils, the values were between 0.06 and 0.08, all within the range corresponding to mature generating rocks. Comparison of the Mor/Hop ratios for the samples with those found in the literature for Venezuelan source-rocks showed that the values for samples 1 (0.7) and 2 (0.5) were within or near the range of values reported by López & Mónaco (2017) for the San Antonio (0.05-0.07) and Naricual (0.58-0.60) rocks.

As the thermal maturity increases, the concentration of the 5α,14α,17α(H)-stigamastane 20S (C_{29}S) biomarker increases, relative to its R isomer. Hence, in thermally immature rocks, the abundance of 5α,14α,17α(H)-stigamastane 20R (C_{29}R) is greater than that of the C_{29}S isomer. The C_{29}R and C_{29}S isomers may be identified using the transition *m/z* 400>217 (Peters et al. 2005b, Waples & Machihara 1991). The values of the C_{29}S/(C_{29}S+C_{29}R) ratio range from 0 to 1, corresponding to indexes from 0 to 100%, with low values indicating less mature oils, while values higher than 0.5 (50%) suggest oils of greater thermal evolution. The values of this ratio for the spill oils collected on the beaches were between 0.28 and 0.53, with the value for sample 3 (0.28) indicating that this oil was less mature, compared to the others. The value for sample 3 was similar to that found for oil D (0.28) from the Sergipe Terra basin. The other oils from the Sergipe-Alagoas basin (A, B, C, and E) presented values between 0.37 and 0.49. Although the values were close, they were indicative of lower maturity, compared to the oils of samples 1, 2, and 4, for which the values ranged from 0.50 to 0.55. From comparison of the C_{29}S/(C_{29}S+C_{29}R) ratio values for the spill oils with those reported in the literature for Venezuelan oils, it could be seen that the value for sample 2 (0.50) was within the range for the Caipe, Silvestre, Sinco, Silván, Palmita, Hato, Mingo, and Maporal basins (0.44-0.50), and was the same as the value for Los Manueles (0.50). In comparison with the source rocks, the values of this diagnostic ratio for samples 1 (0.53), 2 (0.55), and 4 (0.55) were within the range reported for the Naricual rock (0.48-0.58).

In order to support the characterization of immature to mature oils, as well as to reduce possible interferences in the isomerization of steranes caused by factors related to the type of source rock, biodegradation, and weathering, complementary ratios were calculated for C_{29} sterane biomarkers, such as C_{29}ββ(S+R)/(C_{29}ββ(S+R)+C_{29}αα(S+R)). The transition *m/z* 400>217 could be used to identify the biomarkers 5α,14α,17α(H)-stigamastane 20R, 5α,14α,17α(H)-stigamastane 20S, 5α,14β,17β(H)-stigamastane 20R, and 5α,14β,17β(H)-stigamastane 20R (C_{29}ββ(S+R) and C_{29}αα(S+R)), where in which the C_{29}ββ(S+R) isomers are thermally more stable than the $C_{29}\alpha\alpha$(S+R) isomers. An average ratio of approximately 0.7 is obtained for the concentrations at equilibrium (Peters et al. 2005b). For the oil samples collected from the beaches, the values obtained for this ratio were between 0.48 (1) and 0.61 (3), characterizing oils close to the average maturity, with the sample 1 oil being thermally less mature than the others. Relating these values to those obtained for oils from the Sergipe-Alagoas basin showed that the value for crude oil A (0.49) was close to the value obtained for sample 1 (0.48), while the other crude oils B, C, D, and E presented values between 0.31 and 0.42, indicating lower thermal maturity. Comparison of the $C_{29}\beta\beta$(S+R)/($C_{29}\beta\beta$(S+R)+$C_{29}\alpha\alpha$(S+R)) ratio values for the oils collected on the beaches with those reported for Venezuelan oils showed that the value of this ratio for sample 1 (0.48) was in the range described for the Caipe, Silvestre, Sinco, Silván, Palmita, Hato, Mingo, and Maporal oils (45-50%). In the case of the source rocks, only the ratio for sample 1 (0.48) was within the range reported for rocks of Venezuelan origin (Naricual rock: 0.48-0.58) (López & Mónaco 2017).

According to the thermal maturity parameters provided by the diagnostic ratios for the terpane and sterane biomarkers, most of the oils collected on the beaches of Pernambuco and Sergipe originated from rock with equilibrium thermal maturity. In comparison to the samples from the Sergipe-Alagoas basin, the differences in the ratios indicated a lack of similarity between the oils. In contrast, the thermal evolution data for the oil samples collected on the beaches were within the ranges of values described in the literature for source rocks and oils from Venezuelan basins, which could be indicative of similar maturities and origins.

Chemometric analysis

Chemometric analysis of the data for samples 1, 2, 3, 4, A, B, C, D, and E was performed using multivariate statistical tools. Principal component analysis (PCA) (Figure 4) and hierarchical component analysis (Figure 5) were applied to the results obtained for the 27 diagnostic ratios between the oil biomarkers for thermal evolution, depositional environment, and oil-oil correlation (Table III).

From multivariate statistical analysis of the main components, it was possible to evaluate similarities and dissimilarities among the samples (1, 2, 3, 4, A, B, C, D, and E), using the biomarker diagnostic ratios as the input variables. Graphs of the loadings and scores

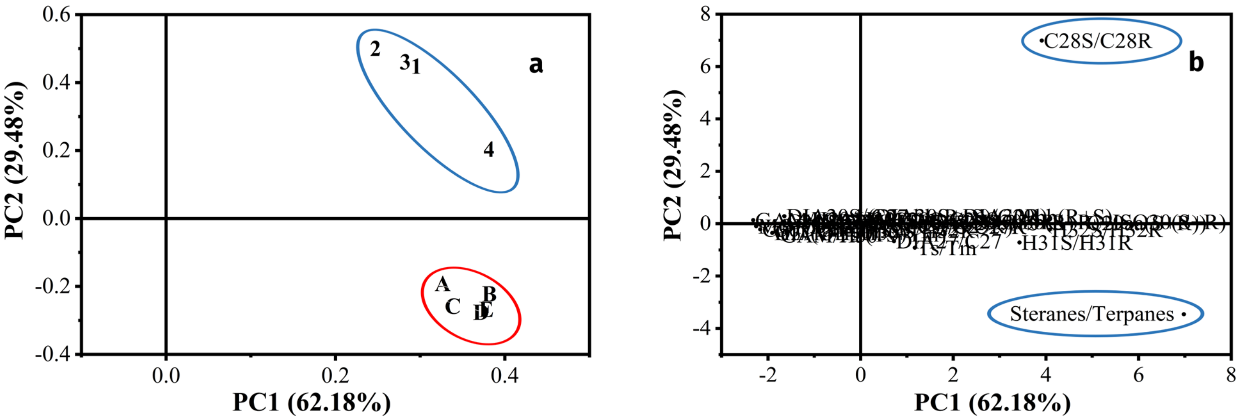

Figure 4. Principal component analysis score and loading plots. (a) Scores plot of PC1 *vs.* PC2, generated from the diagnostic ratios data for the oil biomarkers identified in the samples. (b) Loadings plot of PC1 *vs.* PC2, showing the ratios with the greatest influence on dissimilarity separation.

of principal components 1 and 2 (PC1 and PC2), accounting for 62.18% and 29.48% of the variance, respectively (total cumulative variance of 91.66%), are shown in Figure 4a. Considering PC1, all the samples were positively grouped, while PC2 showed the formation of two distinct groups of samples. The spill oil samples collected from the beaches of Pernambuco and Sergipe (1, 2, 3, and 4) were grouped together, with positive loadings, separated from the oils produced in the Sergipe-Alagoas basin (A, B, C, D, and E), which were grouped with negative loadings. Consequently, the 29.48% data variability explained by PC2 revealed the dissimilarity between these groups of samples. The loadings plot (Figure 4b) showed dispersion of the steranes/terpanes and C28S/C28R diagnostic ratios, with positive loadings in PC1, while in PC2 the C28S/C28R and steranes/terpanes ratios showed positive and negative loadings, respectively. These results demonstrated that the C28S/C28R and steranes/terpanes ratios were mainly responsible for the differentiation of the two groups of samples. The four spill oil samples collected on the beaches of Sergipe and Pernambuco were distinctly different to the five oil samples from the Sergipe-Alagoas basin.

Hierarchical cluster analysis was performed using the data presented in Table III. In the dendrogram (Figure 5), values (y-axis) closer to 0 indicate greater similarity between the samples. Two groups were formed, separating the beach samples (cluster I) from the Sergipe-Alagoas basin oils (cluster II). In cluster I, sample 4 showed a slight dissimilarity in comparison to the other three samples from the same group. This might be attributed to the higher period of exposure to the environment, which might have caused it to undergo a little more influence of weathering processes. However, since the weathering process does not affect too much upon steranes and hopanes, the difference was

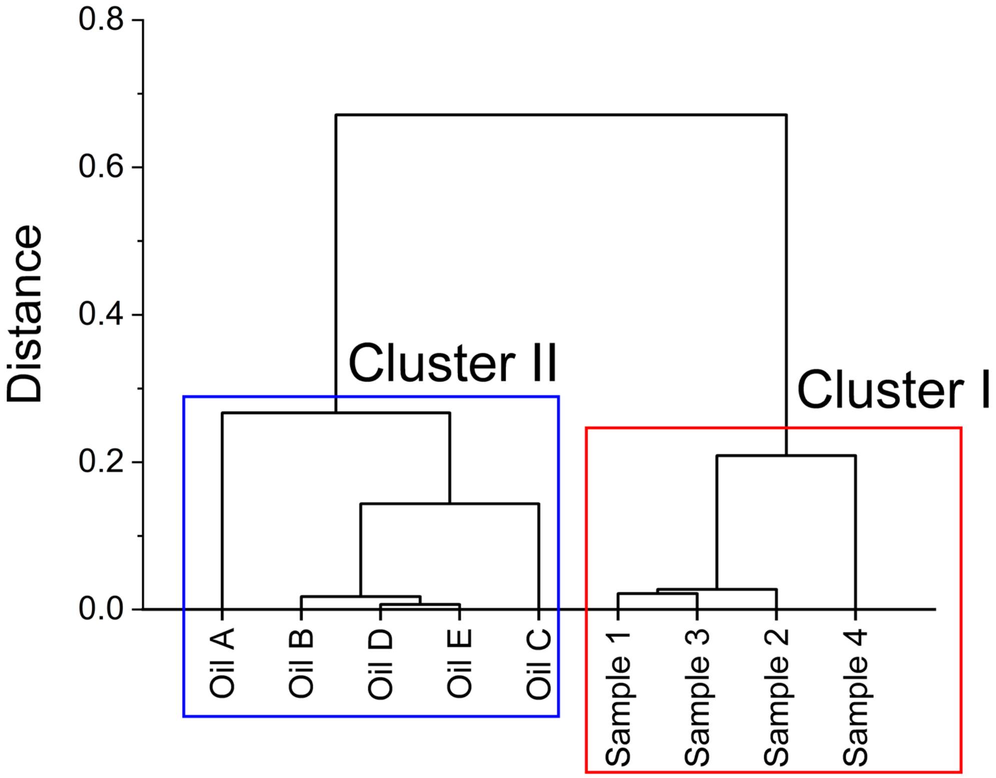

Figure 5. Dendrogram generated from the data for the diagnostic ratios of the samples.

not enough to group it into another cluster (Lima et al. 2021). Overall, the results were in good agreement with the work of Lourenço et al. (2020) and de Oliveira et al. (2020), who reported that the oils reaching the northeast coast of Brazil were from the same incident. The HCA analysis confirmed the dissimilarity between the set of spilled oil samples collected on the beaches of Pernambuco and Sergipe and the samples of oils produced in the Sergipe-Alagoas basin, indicating the improbability that the spill originated from that basin.

Since López & Mónaco (2017) reported ranges, rather than absolute values, for the Venezuelan crude oils and source rocks, it was not feasible to perform multivariate analysis using the Venezuelan data. Nevertheless, among the Venezuelan crude oils and source rocks described by López & Mónaco (2017), the Naricual source rock and the Ayacucho, Los Manueles, La Vicotoria, and Silvestre crude oils showed the greatest similarity to the spill samples, considering the thermal evolution parameters. Taking into account the other parameters, such as the depositional environment and organic matter input diagnostic ratios, as well as the *n*-alkanes profiles, the Naricual formation and the Ayacucho crude oil showed the greatest similarity to the spill samples. Based on the characteristics of the crude oil and the source rocks, reported by Huang et al. (2020), it is known that some of the crude oils from the Orinoco heavy oil belt, including Ayacucho, may derive from the calcareous mudstone of the San Antonio formation or the mudstone of the Naricual formation. Hence, based on the present results, together with the information provided by Huang et al. (2020) and López & Mónaco (2017), Ayacucho was the most likely candidate as the source of the heavy crude oil that reached the northeastern Brazilian coast in 2019.

CONCLUSIONS

These results corroborated recently published information concerning the oil that reached the northeastern Brazilian coast on August 30, 2019, indicating that all the spilled oil samples came from the same source. The possibility that oils from the Sergipe-Alagoas basin could have contributed to the disaster was dismissed after evaluation of the profiles of *n*-alkanes, hopanes, and steranes in the petroleum materials collected from the beaches. Among other factors, the presence of the highly specific biomarker 18α(H)-oleanane could be highlighted. Comparison with diagnostic ratio values and *n*-alkanes distribution patterns reported in the literature for Venezuelan source rocks and oils containing 18α(H)-oleanane revealed many similarities with the oil samples collected on the beaches. Among the Venezuelan oils, we suggest that Ayacucho oil, from Naricual Formation in Venezeluan Eastern basin was the most likely candidate as the source of the weathered oil that reached the northeastern coast of Brazil in 2019.

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SUPPLEMENTARY MATERIAL

Figures S1-S4.

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