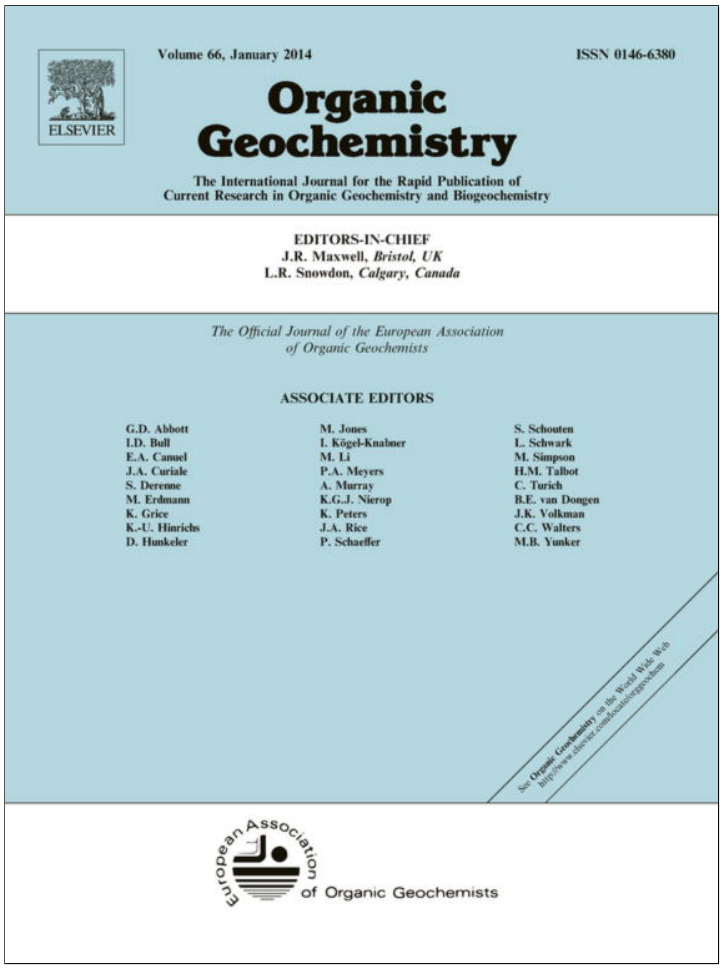

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Study of the biodegradation levels of oils from the Orinoco Oil Belt (Junin area) using different biodegradation scales

Liliana López *

Instituto de Ciencias de la Tierra, Facultad de Ciencias, Universidad Central de Venezuela, 3895, Caracas 1010 A, Venezuela

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ABSTRACT

This work presents a study of the molecular composition of the saturated and aromatic hydrocarbon fractions of crude oils from the Orinoco Oil Belt (Junín area) in the Eastern Venezuelan Basin, with the purpose of classifying these samples following two distinct biodegradation assessment schemes: the PM scale [Peters, K.E., Moldowan, J.M., 1993. The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments. Prentice Hall, Englewood Cliffs, New Jersey, p. 363] and the Manco scale [Larter, S., Huang, H., Adams, J., Bennett, B., Snowdon, L.R., 2012. A practical biodegradation scale for use in reservoir geochemical studies of biodegraded oils. Organic Geochemistry 45, 66–76]. Both scales agree on the presence of different levels of biodegradation for the analyzed oils, although they are based on different groups of compounds. The PM scale uses mainly compounds from the saturated hydrocarbon fractions (e.g., *n*-alkanes, acyclic isoprenoids, terpanes and steranes) as well as aromatic steroids. On the other hand, the Manco scale considers other compounds (e.g., alkyltoluenes, naphthalene, methylnaphthalene, phenanthrene, alkylphenanthrenes and methyldibenzothiophenes) not included in the PM biodegradation scale. Thus, the combined use of these two scales allows the determination of the level of biodegradation of both saturated and aromatic compound classes. Dibenzothiophene (DBT), which was not included for the Manco score determination, also shows variations in peak intensity when compared to C_4-alkylnaphthalenes, presumably associated with the process of biological alteration. The differences in the biodegradation levels observed in the present study may be attributed to variations in parameters that control biodegradation rates laterally across the study area or the existence of varying communities of microorganisms, among other possible factors.

1. Introduction

Organic geochemistry has incorporated several techniques and processes to support oil exploration and production. Analyses of source rocks, rock extract (bitumen) and crude oils provide information on organic matter type, paleoenvironmental sedimentation conditions, source rock maturity and, if possible, oil–oil and oil–source rock correlation. The composition of unaltered crude oils is primarily determined by the characteristics of the source rock: (1) organic matter type, (2) depositional environment and (3) level of maturity. In addition, hydrocarbon reservoirs may be subject to multiple stages of charge, as well as to a number of alteration processes (e.g., biodegradation, water washing, deasphalting, gravity segregation and reservoir thermal alteration), that also contribute to changes in oil composition (Volkman et al., 1983a,b, 1984; Tissot and Welte, 1984; Hunt, 1995; Wenger et al., 2002; Peters et al., 2005; Larter et al., 2012). All of these factors result in a wide range of variability in oil quality that can mislead the interpretation of geochemical analyses; for example, in correlation studies (Curiale, 2008).

Biodegradation of oil involves the competing microbial metabolism of various classes of compounds that alters oil fluid properties and economic value. More specifically, oil biodegradation typically (a) decreases API gravity, (b) reduces the content of saturated and aromatic hydrocarbons relative to polar compounds (c) increases oil viscosity, (d) increases oil acidity and (e) increases the sulfur content, and the concentration of certain metals (e.g., V and Ni). As a consequence, the residual oil resulting from biodegradation becomes enriched in NSO compounds (resin and asphaltene fractions), sulfur and metals (Wenger et al., 2002; Peters et al., 2005; Larter et al., 2006). The alteration of petroleum by biodegradation is controlled by reservoir temperature (< 80 °C), nutrients (nitrogen, potassium and phosphorus), oil composition, the oil–water contact area, water salinity and oil volume in the reservoir (Wenger et al., 2002; Larter et al., 2006). Some biomarkers are of limited use when oils show evidence of heavy to severe biodegradation (Peters et al., 2005). Therefore, other parameters must be used in correlation studies; for example, the V/Ni ratio in oils (Al-Shahristani and Al-Thyia, 1972; López et al., 1991, 1995;

* Tel.: +58 212 6051417; fax: +58 212 6051152.
E-mail address: liliana.lopez@ciens.ucv.ve

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Frankenberger et al., 1994; Alberdi et al., 1996; López and Lo Mónaco, 2004, 2010).

The extent of biodegradation has been evaluated using different scales based on the alteration/removal of saturated and aromatic compounds. For example, Volkman et al. (1983b) developed a biodegradation scale based on the alteration of compounds in the saturated hydrocarbon fractions. Later, Volkman et al. (1984) expanded this scale to include aromatic hydrocarbons. The PM biodegradation scale (Peters and Moldowan, 1993) is based on saturated biomarkers and select aromatic compounds (mainly aromatic steroids). Another scale that considers both saturated and aromatic hydrocarbons was developed by Wenger et al. (2002).

Therefore, different biomarker biodegradation scales have been developed to assess the extent of oil biodegradation based on the comparisons of the relative abundance of a selection of hydrocarbon compound classes in saturated (e.g., *n*-alkanes, pristane, phytane, terpanes, steranes) and aromatic (e.g., C_1-, C_2-, C_3- and C_4-naphthalenes, C_2-phenanthrenes, and aromatic steroids) hydrocarbon fractions with different susceptibility to biodegradation (Volkman et al., 1983b, 1984; Peters and Moldowan, 1993; Wenger et al., 2002).

In the aromatic compounds, the biodegradation level decreases with increasing number of aromatic rings, and isomers within individual compound classes have different susceptibility to biodegradation related to the position of the alkyl substituents (Huang et al., 2004a). Based on the study of crude oils from the Barrow sub-basin (Western Australia), Volkman et al. (1984) proposed the following order of biodegradation of aromatic hydrocarbons in oxic conditions: benzene > toluene > naphthalene > phenanthrene ⋙ polycyclic aromatic hydrocarbons; and there is a C_1 -> C_2 > C_3 > C_n (*n* is the total number of C atoms in the alkyl substituent) tendency to biodegradation within each class. From the relative concentration of alkylnaphthalenes, the following order of biodegradation rate was proposed: 2MN > 1MN > 2,7-, 1,7- and 1,6-DMN > 1,3- and 2,6-DMN > 1,4- and 1,5-DMN > 1,2-DMN > 2,3-DMN and 1-EN > 2-EN > 1,8-DMN (MN = methylnaphthalene, DM = dimethylnaphthalene and EN = ethylnaphthalene). With respect to the selective biodegradation of structural isomers, it is considered that the β-methyl substituents are oxidized faster than the α-methyl substituent.

Recently, Larter et al. (2012) developed a new biodegradation scale called Manco (*Modular Analysis and Numerical Classification of Oils*) scale, based on integrating the extent of biodegradation of various aromatic compounds such as alkyl aromatic compounds (e.g., alkyltoluenes, naphthalenes, methylnaphthalenes, phenanthrene, methylphenanthrenes, methyldibenzothiophenes) and steranes. The authors noted that this scale can be applicable to heavily and severely biodegraded oils from western Canada, with biodegradation PM levels 4–8, as well as to the large reserves of biodegraded, heavy and extra heavy crude oils located in the Orinoco Oil Belt from eastern Venezuela.

It is well known that crude oils from the Orinoco Oil Belt are heavy and extra heavy due to biodegradation (Cassani and Eglinton, 1986; Fiorillo, 1987; Audemard et al., 1987; Alberdi et al., 1996). However, the degree of biodegradation of these oils varies considerably; some oils are characterized by the presence of *n*-alkanes with an unresolved complex mixture (UCM) without sterane and hopane alteration and others show alteration of steranes and hopanes with formation of 25-norhopanes (López and Lo Mónaco, 2010). The level of biodegradation influences the enhanced oil recovery process. This article presents the geochemical analysis of eleven crude oils from the Orinoco Oil Belt (Junín area) to determine their biodegradation levels using both PM and Manco scales. The application of some geochemical parameters as indicators of organic matter type, depositional environment and level of maturity of source rock is discussed to corroborate that the observed changes are the result of biodegradation.

2. Geological setting

The Eastern Venezuela Basin covers an area of 165,000 km^2, corresponding to a number of fields located in the northern part and the Orinoco Oil Belt within the southern flank of the basin (Fig. 1). This basin is divided into the Guárico and the Maturin sub-basins. The area under study is located in the Maturin sub-basin (González de Juana et al., 1980).

The Orinoco Oil Belt is divided into four areas: Boyacá (Machete), Junín (Zuata), Ayacucho (Hamaca) and Carabobo (Cerro Negro). The area under study is located in the Junín area. Stratigraphically, it is made up of rocks from Paleozoic and Cenozoic age that overlie the Precambrian igneous–metamorphic complex of the Guayana Shield. The principal hydrocarbons accumulations are in the basal sands of the Oficina (early Miocene) and the Merecure (Oligocene) formations, with some minor oil accumulations in the underlying sands of the Temblador Group (Cretaceous) (Vega and Rojas, 1987). The oil reservoirs are characterized by fluvial sequences with high sand content and fluvio-deltaic environments with low sand proportions (Kopper et al., 2001; Pardo et al., 2007). The biodegraded hydrocarbon accumulations in the basin are heavy to extra heavy, with high sulfur concentration (Alberdi et al., 1996; López and Lo Mónaco, 2010). Based on the V/Ni ratio and some biomarkers, the crude oils from the Orinoco Oil Belt have been separated into three families (Alberdi et al., 1996). Two oil families have high V/Ni ratios (A: V/Ni = 3.6 and B: V/Ni = 5.2) with the presence of 18α(H)-oleanane in oils of family A. These two oil families originated from different facies of the source rock in the Upper Cretaceous Querecual and the San Antonio formations (Guayuta Group). Analyses of biomarker parameters and V/Ni ratio in these oils indicate that they originated from a carbonate rich source rock facies, with marine organic matter input deposited in more reducing conditions. The third oil family has a low V/Ni ratio (C: V/Ni = 0.7) and contains 18α(H)-oleanane and high diasteranes, indicating that the oils originated from an Upper Cretaceous or Tertiary deltaic marine shale (Alberdi et al., 1996).

3. Experimental

3.1. Analytical procedure

Eleven crude oil samples (labeled A through K) were separated into asphaltene and maltene fractions applying the NF-T60.115 method (AFNOR, 1975). The maltene fraction was separated into its saturated, aromatic and resin fractions by means of adsorption chromatography, using packed columns (20 cm long and 1.5 cm in diameter) with alumina as the stationary phase (20 g). The saturated hydrocarbons were eluted with *n*-hexane (30 ml), the aromatic hydrocarbons with toluene (20 ml), and a mixture (15 ml) of toluene:methanol (70:30) was used for the elution of polar compounds (resins). Purification of the saturated and the aromatic hydrocarbon fractions was carried out twice by liquid chromatography using packed columns like those described above.

3.2. Samples analysis

Gas chromatography (GC) of the saturated hydrocarbon fractions was carried out on a 6890N Agilent technologies gas chromatograph using a flame ionization detector (FID) and type DB-1 fused capillary columns (60 m × 0.25 mm × 0.25 μm). Detailed analyses of the saturated and aromatic biomarkers were performed

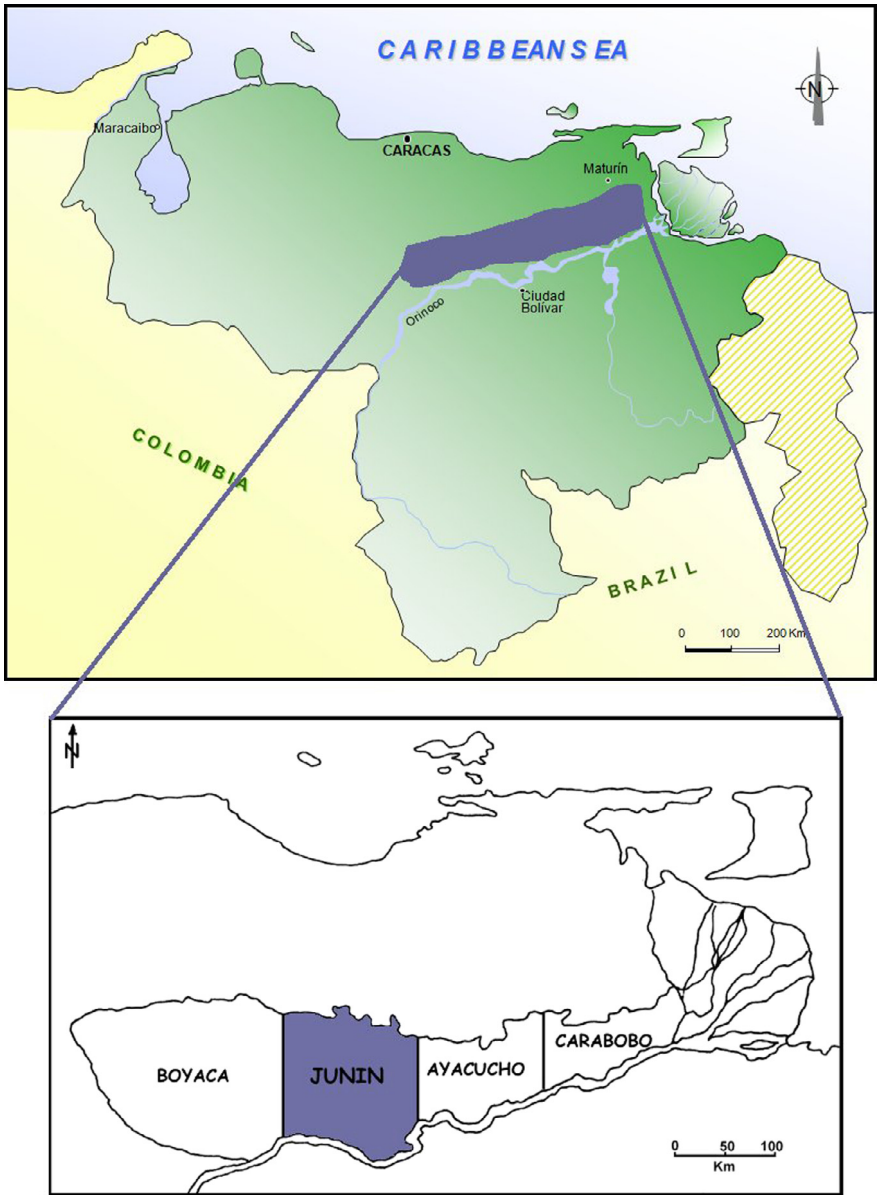

Fig. 1. Location of the Junín area in the Orinoco Oil Belt, Venezuela (after Fiorillo, 1987).

by gas chromatography–mass spectrometry (GC–MS) coupling the gas chromatograph to a 5975 Agilent Technologies mass spectrometer (operated in the single ion monitoring mode). In this case, the GC system was equipped with either type DB-1 or DB-5, fused silica capillary column (60 m × 0.25 mm × 0.25 μm) to analyze the saturated and aromatic fractions. The monitored ions were: m/z = 191, 177, 217, 218 for terpanes, steranes and diasteranes; m/z = 178, 192, 184, 198 for phenanthrene, methylphenanthrenes, dibenzothiophene + tetramethylnaphthalenes, and methyldibenzothiophenes + pentamethylnaphthalenes, respectively; and m/z = 231 for triaromatic steroids.

4. Results and discussion

4.1. Oil composition

All the oils extracted from shallow depth reservoirs are heavy and extra heavy with API gravities ranging from 7.3–9.2 (Table 1). They have a high content of resins and asphaltenes (NSO: 49–75%), and low content of saturated hydrocarbons (5–11%) as shown in Fig. 2.

4.2. Biomarkers: Organic matter type and source rock

Considering that the distribution of terpanes appears unaltered by the biodegradation process that affected the investigated oils, these biomarkers are used in this work to assess the characteristics of the source rock(s) that generated these oils. Detailed study of biomarkers in terpanes mass chromatogram (m/z = 191) indicated the presence of 18αH-oleanane (Figs. 3–5) and the oleanane index (OI = [oleanane/(oleanane + C_{30} hopane] × 100) was calculated. The values obtained range from 3.4–10.3 (Table 2), all related to limited higher plant input during organic matter deposition (Peters et al., 2005). This suggests variations in terrigenous organic material input during deposition of the source rock. Of the three families of crude oils defined by Alberdi et al. (1996) for the Orinoco Oil

Table 1
Depth (m), API gravity and SARA oil composition (%w/w) for oil samples from Junín area.

Sample	Depth	API	Saturated	Aromatic	Resins	Asphaltenes	NSO
A	[a]	8.5	5	26	54	15	69
B	372.2	8.4	7	24	57	12	69
C	357.6	7.3	8	21	60	11	71
D	412.7	8.7	6	31	53	10	63
E	316.4	8.4	11	14	67	8	75
F	427.4	9.0	5	30	50	15	65
G	439.9	8.3	6	31	58	5	63
H	340.2	9.0	6	27	56	11	67
I	324.0	9.2	6	30	54	10	64
J	337.8	9.3	10	40	38	12	50
K	323.8	8.4	6	45	36	13	49

[a] Data not available.

Belt, the crude oils from the Junín area (formerly Zuata) lie within the families A and B. However, the presence of 18αH-oleanane was reported only in oils of family A, with an average oleanane index of 5%. Therefore, it is proposed here that the analyzed crude oils are related to the oils of family A previously studied by Alberdi et al. (1996).

The C_{24}/C_{23} and C_{26}/C_{25} tricyclic terpanes and $C_{31}R/C_{30}$ hopane ratios can be used to distinguish oils derived from carbonate, marine shale, lacustrine, marls or carbonate source rocks (Peters et al., 2005). The calculated ratios are C_{24}/C_{23} = 0.42–0.49, C_{26}/C_{25} = 0.75–1.76 (high value only oil A), and $C_{31}R/C_{30}$ hopane = 0.39–0.44 (Table 2). According to Peters et al. (2005), oils from carbonate source rocks can be distinguished by low C_{24}/C_{23} ratio (< 0.6), low C_{26}/C_{25} ratio (C_{26}/C_{25} < 1.1) and $C_{31}R/C_{30}$ hopane ratios > 0.30. Therefore, the results for the C_{24}/C_{23}, C_{26}/C_{25} and $C_{31}R/C_{30}$ hopane ratios, along with the low diasterane to sterane signal, suggest that the analyzed oils originated from a marine-carbonate source rock.

Additional information on source rock lithofacies is derived from the isomer distribution pattern of methyldibenzothiophenes (MeDBT) (Hughes, 1984). The distribution pattern of DBT isomers corresponds to 4-MeDBT > 2,3-MeDBT < 1-MeDBT, as presented in Fig. 6. The data show that these oils are associated with carbonate rich source rocks, as already indicated by the tricyclic terpanes and hopane ratios (although caution must be exercised when using these parameters, since they may be affected by biodegradation).

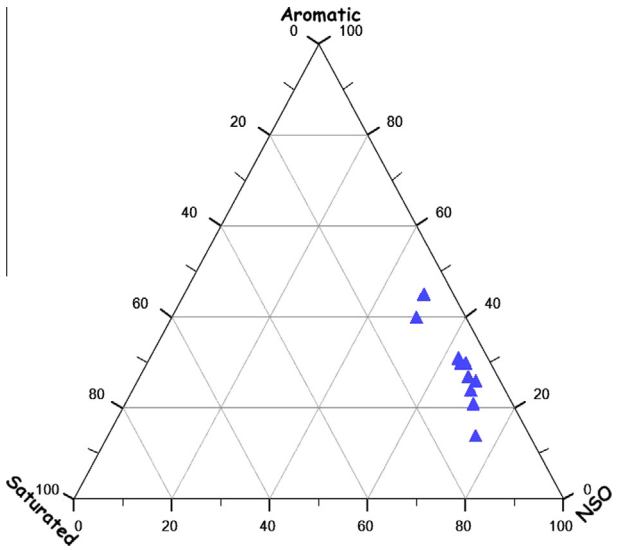

Fig. 2. SARA compositions of oils.

4.3. Biomarkers: Maturity

The Ts/(Ts + Tm) ratio is a thermal maturity parameter based on the lower thermal stability of 17α-22,29,30-trisnorhopane (Tm) compared to that of 18α-22,29,30-trisnorneohopane (Ts). The calculated values for Ts/(Ts + Tm) ratio for the present samples (Table 2) were low (0.17–0.26). According to Peters et al. (2005), oils from carbonate source rocks show unusually low Ts/(Ts + Tm) ratio (⩽ 0.25) compared to those from shales of anoxic environments [Ts/(Ts + Tm) = 0.26–0.35]. The results in Table 2 indicate small differences in the level of maturity among the oil samples from the Junín area.

The C_{31} to C_{35} homohopane isomerization calculated by the ratio 22S/(22S + 22R) presented values in the range of 0.54–0.70 (Table 3) indicating that the main phase of oil generation has been reached or surpassed. Other indicators of maturity, such as steranes isomerization (C_{29}20S or C_{29}ββ), cannot be used, because the oils are biodegraded and the microbial alteration affects the distribution of regular steranes. Instead, other maturity parameters based on aromatic hydrocarbons are used and were applied in this work. Therefore, the aromatic distributions obtained for phenanthrene (P, m/z = 178), methylphenanthrenes (Me-P, m/z = 192) and triaromatic steroid (TAS, m/z = 231), which are not altered by biodegradation, were analyzed for the identification of oil maturity (Table 2).

The maturity of oils was also interpreted using the inferred vitrinite reflectance (%Rc, equation in Table 2) (Radke and Welte, 1983; Radke et al., 1986) derived from the methylphenanthrene index (MPI-1), which is calculated using the peak areas of P and Me-P from m/z = 178 and 192 fragmentograms (Fig. 6), respectively. According to the values of these parameters, the samples from Junín area are in the beginning of the oil window. However, caution needs to be exercised when using this relationship in crude

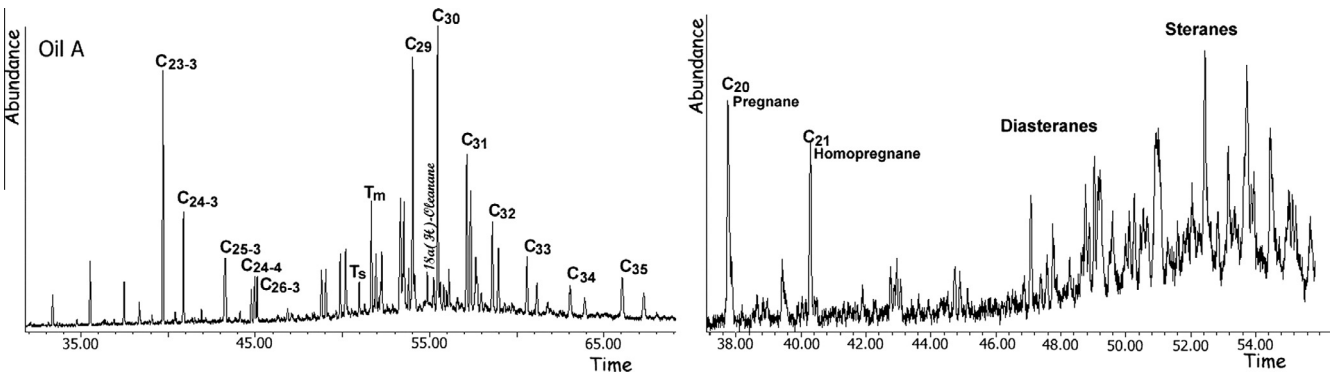

Fig. 3. Mass chromatograms of the terpanes (m/z 191, left) and steranes (m/z 217, right) in the saturated hydrocarbon fractions isolated from Oil A.

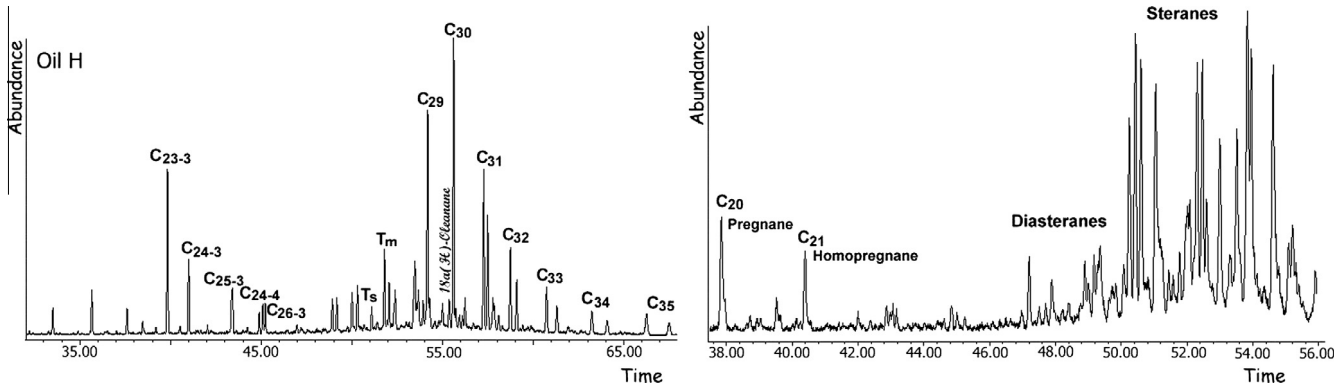

Fig. 4. Gas chromatograms of the distribution of terpanes (m/z 191, left) and steranes (m/z 217, right) in the saturated hydrocarbon fractions extracted from Oil H.

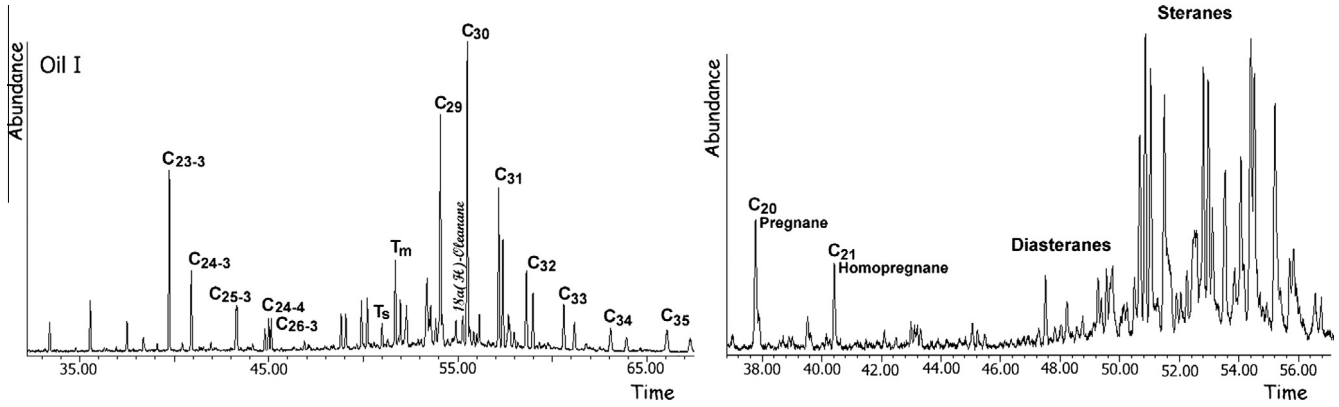

Fig. 5. Gas chromatograms of the distribution of terpanes (m/z 191, left) and steranes (m/z 217, right) in the saturated hydrocarbon fractions extracted from Oil I.

Table 2
Molecular ratios for oils from the Junín area.

Sample	$C_{24\text{-}3}/C_{23\text{-}3}$[a]	$C_{26\text{-}3}/C_{25\text{-}3}$[a]	$C_{31}22S/(22S+22R)$[a]	Ts/(Ts + Tm)[a]	Oleanane index[a]	MP1-1[b]	Rc[b]	TAS[a]
A	0.42	1.76	0.58	0.19	10.3	0.79	0.88	14.0
B	0.47	0.78	0.58	0.26	6.7	0.75	0.85	16.0
C	0.43	0.78	0.58	0.19	8.3	0.91	0.95	12.4
D	0.46	0.76	0.59	0.23	6.3	1.04	1.02	12.3
E	0.49	0.79	0.58	0.26	8.5	0.80	0.88	12.1
F	0.45	0.77	0.59	0.18	3.4	0.73	0.84	13.4
G	0.45	0.78	0.59	0.17	3.6	NA	NA	NA
H	0.47	0.75	0.58	0.20	8.3	0.90	0.94	13.9
I	0.47	0.85	0.58	0.21	6.9	NA	NA	NA
J	0.47	0.88	0.58	0.26	8.5	0.94	0.96	15.5
K	0.44	0.83	0.58	0.19	10.3	1.09	1.05	13.8

$C_{24\text{-}3}/C_{23\text{-}3}$: Terpane tricyclic C_{24} to C_{23} ratio.
$C_{26\text{-}3}/C_{25\text{-}3}$: Terpane tricyclic C_{26} to C_{25} ratio.
Homohopanes isomerization = $C_{31}22S/(22S + 22R)$.
Ts/Ts + Tm: [$C_{27}18\alpha$-trisnorhopane/($C_{27}18\alpha$-trisnorhopane + $C_{27}17\alpha$Trisnorhopane)] × 100.
Oleanane index = oleanane/(oleanane + C_{30} hopane) * 100.
MP1-1 = 1.5(2Me + 3Me)/(P + 1Me + 9Me).
Rc = 0.6MPI-1 + 0.4.
TAS = TAI/(TAI + TAII) = ((C_{20}–C_{21})/(C_{26}–C_{28})) × 100; TAI = group I of triaromatic steroids C_{20}–C_{21}; TAII = group II of triaromatic steroids C_{26}–C_{28}.
NA: no analyzed.
[a] Peters et al. (2005).
[b] Radke and Welte (1983).

oils from marine origin (Radke and Welte, 1983; Radke et al., 1986).

Fig. 6 displays the mass chromatogram of triaromatic steroid hydrocarbons (m/z = 231) for the oil sample A. According to the literature, the TAS remains unaltered in biodegraded oils and is used to determine oil source maturity level (Volkman et al., 1984). The study is based on the conversion of group II C_{26}–C_{28} (TAS-II) to group I C_{20}–C_{21} (TAS-I) triaromatic steroids (Peters and Moldowan, 1993), by side chain cleavage during thermal maturation (equation in Table 2). The calculated ratios TAS = TAS-I/(TAS-I + TAS-II), indicate low conversion of TAS-II to TAS-I, and therefore a low maturity level for studied oils.

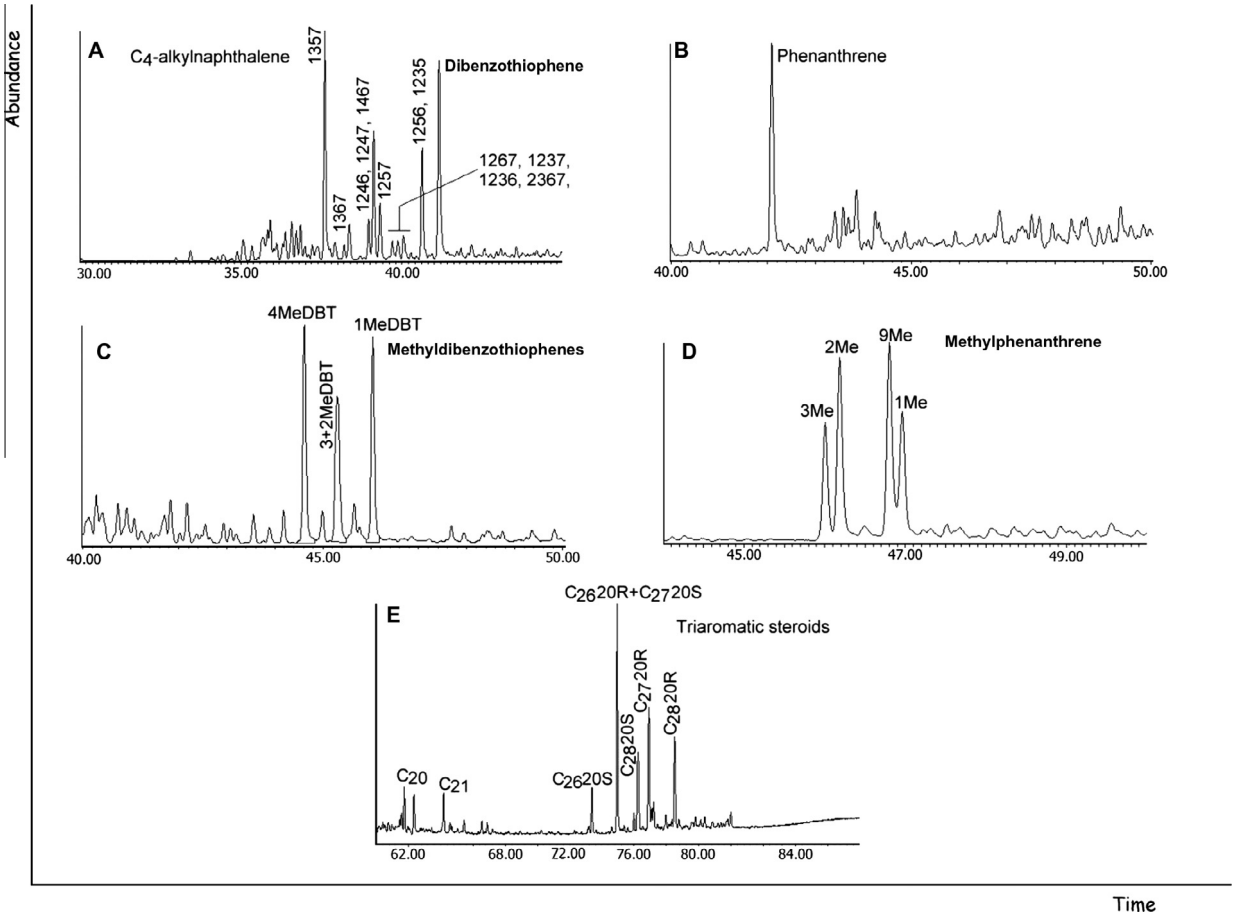

Fig. 6. Gas chromatograms of the: (A) C4-alkylnaphthalenes + dibenzothiophenes (*m*/*z* 184), (B) phenanthrenes (*m*/*z* 178), (C) methyldibenzothiophenes (*m*/*z* 198), (D) methylphenanthrene (*m*/*z* 192) and (E) triaromatic steroids (*m*/*z* 231) for Oil A.

Table 3
Manco scores calculated for oils from Orinoco Oil Belt Venezuela, Junín area.

Sample	Alkyl-tol	N + MN	C2N	C3N	MeDBT	C4N	C0-2P	Sterane	MN2	PM level
A	4	4	4	4	0	2	0	3	963	6–7 Heavy
B	4	4	4	4	0	0	0	0	500	4–5 Moderate
C	4	4	4	4	0	0	0	1	876	5–6 Moderate to heavy
D	4	4	4	4	0	3	0	0	716	4–5 Moderate
E	4	4	4	4	0	0	0	1	876	5–6 Moderate to heavy
F	4	4	4	4	0	0	0	1	876	5–6 Moderate to heavy
G	4	4	4	4	0	0	0	1	500	4–5 Moderate
H	4	4	4	4	0	1	0	1	500	4–5 Moderate
I	4	4	4	4	0	3	0	1	884	5–6 Moderate to heavy
J	4	4	4	4	0	3	0	1	884	5–6 Moderate to heavy
K	4	4	4	4	0	0	0	1	876	5–6 Moderate to heavy

Alkyl-tol: alkyl toluenes, N + MN: $C_{0\text{-}1}$ naphthalenes, C2N: C_2-naphthalenes, C3N: C_3-naphthalenes, MeDBT: methyl dibenzothiophenes, C4N: C_4-naphthalenes, C0-2P: $C_{0\text{-}2}$ phenanthrenes.

4.4. Biomarkers: biodegradation level: PM scale

Fig. 7 shows typical examples of the distribution of *n*-alkanes and acyclic isoprenoid alkanes for two representative oils labeled A and C. All the samples studied exhibited a similar distribution for these compounds. The observed depletion of *n*-alkanes and acyclic isoprenoid alkanes (phytane and pristane) indicate that all oils studied are biodegraded. In addition, the analysis of the distribution of terpanes and steranes revealed different levels of biodegradation according to the PM scale, as discussed next.

Oil A, was biodegraded to PM level 6–7 (heavy) as indicated by the depletion of *n*-alkanes and acyclic isoprenoid alkanes (pristane and phytane) and the alteration of C_{27}–C_{29} steranes. There is evidence of partial degradation of C_{29}–C_{30} hopanes and C_{31}–C_{35} homohopanes. The analysis of the fractions derived from oil A also revealed a preservation of diasteranes, C_{20} pregnane and C_{21} homopregnane, as presented in Fig. 3.

Oils C, E, F, H, J and K have PM level 5–6 (moderate to heavy) and are characterized by depleted *n*-alkanes and acyclic isoprenoid alkanes (pristane and phytane), abundance of C_{23} tricyclic terpanes and slightly altered steranes. There is evidence of partial degradation of C_{29}–C_{30} hopanes and C_{31}–C_{35} homohopanes (Fig. 4).

Oils B, D, G and I have a PM level 4–5 (moderate) and are characterized by depleted *n*-alkanes and acyclic isoprenoids (pristane

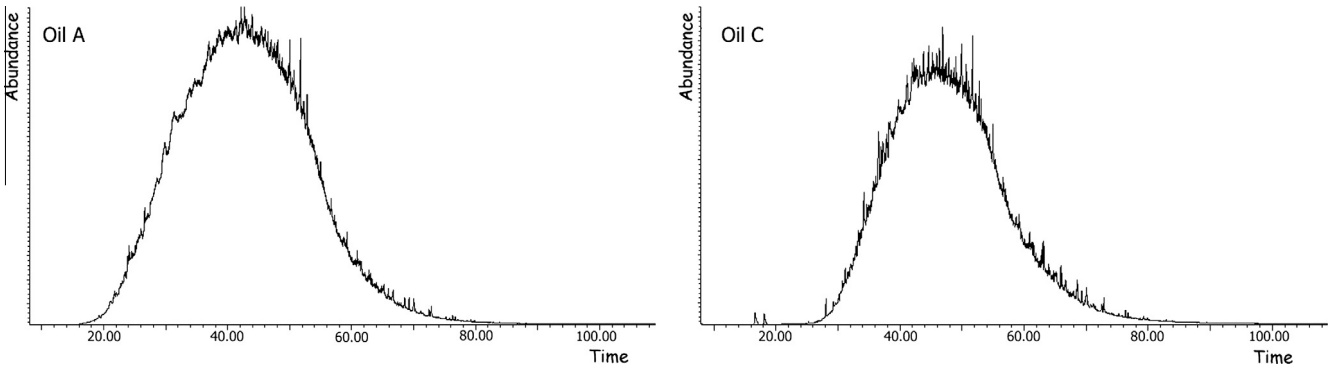

Fig. 7. Gas chromatogram showing the typical distribution of *n*-alkanes in the saturated hydrocarbon fractions of two representative crude oils.

and phytane), and abundance of C_{23} tricyclic terpane. There is no evidence of alteration of C_{27}–C_{29} steranes, but the C_{29}–C_{30} hopanes and C_{31}–C_{35} homohopanes are partially biodegraded (Fig. 5).

At high levels of biodegradation, homohopanes may be transformed into 25-norhopanes (10-demethylated hopanes) (Peters et al., 1996, 2005). These compounds apparently result from the bacterial removal of the methyl group at C-10 from the regular hopanes and are identified using the ion $m/z = 177$ mass chromatogram. When the formation of 25-norhopanes occurs from hopanes, its appearance in the $m/z = 177$ chromatograms is observed at lower retention times (Volkman et al., 1983b). Comparison of the $m/z = 191$ and $m/z = 177$ mass chromatograms (Fig. 8) reveals variation in the hopanes retention times. That is, there is evidence of biodegradation due to the loss of a methyl group, which indicates the presence of 25-norhopanes at trace levels compared to hopanes.

4.5. Biomarkers: biodegradation level: Manco scale

The analyzed oils are characterized by the preservation of phenanthrene, methylphenanthrenes, C_4-alkylnapthalenes, dibenzothiophene (DBT), methyldibenzothiophenes (MeDBT) and aromatic steroids (Fig. 6), as well as the depletion of alkyltoluenes, naphthalene, methylnaphthalenes and C_2–C_3 alkylnaphthalenes. The alteration of naphthalenes follows the order C_0- > C_2- > C_3- > C_4-naphthalene proposed by Wenger et al. (2002). The presence of terpanes, steranes and aromatic steroids indicates that the crude oils have not reached a biodegradation level of PM 10.

The distribution of phenanthrene, methylphenanthrenes, MeDBT and triaromatic steroids are similar in all analyzed samples, without variations in the relative intensities of these compounds among samples. However, DBT and C_4-alkylnapthalenes exhibit variation in their relative distributions (Fig. 9), allowing the determination of variations in the biodegradation degree of oils according to the Manco scale. With respect to the susceptibility to biodegradation of the methyl isomers, the β-methylnaphthalenes are oxidized faster than the α-methylnaphthalenes (Volkman et al., 1984). Therefore, the C_4-naphthalene (TeMN) isomers most susceptible to biodegradation would be 1,3,6,7-TeMN (αβββ); 1,2,3,6-TeMN (αβββ); 2,3,6,7-TeMN (βββ) and 1,2,3,7-TeMN (αβββ), whose signal intensities are lower in crude oils A, D, H, and J that present alteration of these compounds (see Fig. 9 and Table 3). In contrast, the β isomers are more thermally stable than their α counterparts (Radke, 1987). However, it has been indicated that the β isomers are not always more abundant in mature crude oils; especially at high methylation levels (e.g., in C_3- and C_4-naphthalenes). On the other hand, it was previously concluded that thermal stress is the main contributing factor to the distribution of these compounds, which affects each class of methylated naphthalene in a very similar manner (van Aarssen et al., 1999). Because the analyzed crude oils are approximately the same thermal maturity, it is suggested that biodegradation is the main contributing factor to the distribution of C_4-naphthalenes.

Table 3 shows the assignment of Manco scores for the determination of the Manco number (MN2) in the Manco scale, based on the varying degrees of alteration by biodegradation of mono- and poly-aromatic hydrocarbons and steranes in heavy and extra heavy crude oils from the Orinoco Oil Belt. This scale provides new information on the biodegradation level of these oils and includes other compounds different from saturated hydrocarbons (*n*-alkanes, acyclic isoprenoids, steranes and hopanes) and some aromatics (monoaromatic and triaromatic steroids), used in the PM scale. Eight compound classes are used in this work to define the biodegradation level based on the Manco scale, i.e., 1-, 2- and 3-ring aromatic hydrocarbons (e.g., alkyltoluenes, naphthalenes + methylnaphthalenes, C_2-, C_3-, C_4-alkylnaphthalenes, C_0–C_2 alkylphenanthrenes), MeDBT and steranes (Larter et al., 2012). The scores, ranging from Manco score MS = 0–4, distinguish qualitatively five levels of biodegradation for each compound class as: non-degraded or pristine (MS = 0), very slightly degraded or light (MS = 1), midway between the extremes or moderate (MS = 2), not quite fully degraded or heavy (MS = 3), and fully degraded or depleted (MS = 4). The MS values obtained for a given sample are then used to derive the biodegradation level descriptor, known as Manco number (MN2), set to an arbitrary maximum value of 1000.

The detailed study of the group of compounds used to determine the MN2 values indicates that only C_4-alkylnaphathalenes, DBT (not considered in the MN2 determination), and steranes show differences in the biodegradation levels. Fig. 9 shows the distribution of C_4-alkylnaphthalenes and DBT ($m/z = 184$), for selected samples. Clear variations are observed in the relative intensities of both C_4-alkylnaphthalenes and DBT. This suggests differences in the alteration level of these compounds in the analyzed oils. Although DBT is more resistant to biodegradation compared to its methylated isomers (Huang et al., 2004a), here there are variations in the intensity of the DBT and C_4-alkylnaphthalene signals (Fig. 9). This suggests differences in the alteration level of these compounds in the analyzed oils. For example, bacterial communities have been shown to degrade DBT (Frassinetti et al., 1998; Lu et al., 1999; Seo et al., 2006).

The oil with the highest Manco number (MN2 = 963) is the most altered with heavy biodegradation level according to the PM scale as well. The oils exhibiting moderate PM biodegradation level have MN2 numbers < 716, and those ranked in the moderate to heavy PM level present MN2 from 876–884 (Table 3). Although these crude oils are ranked at similar biodegradation levels according to both the PM and Manco scales, the latter provides additional

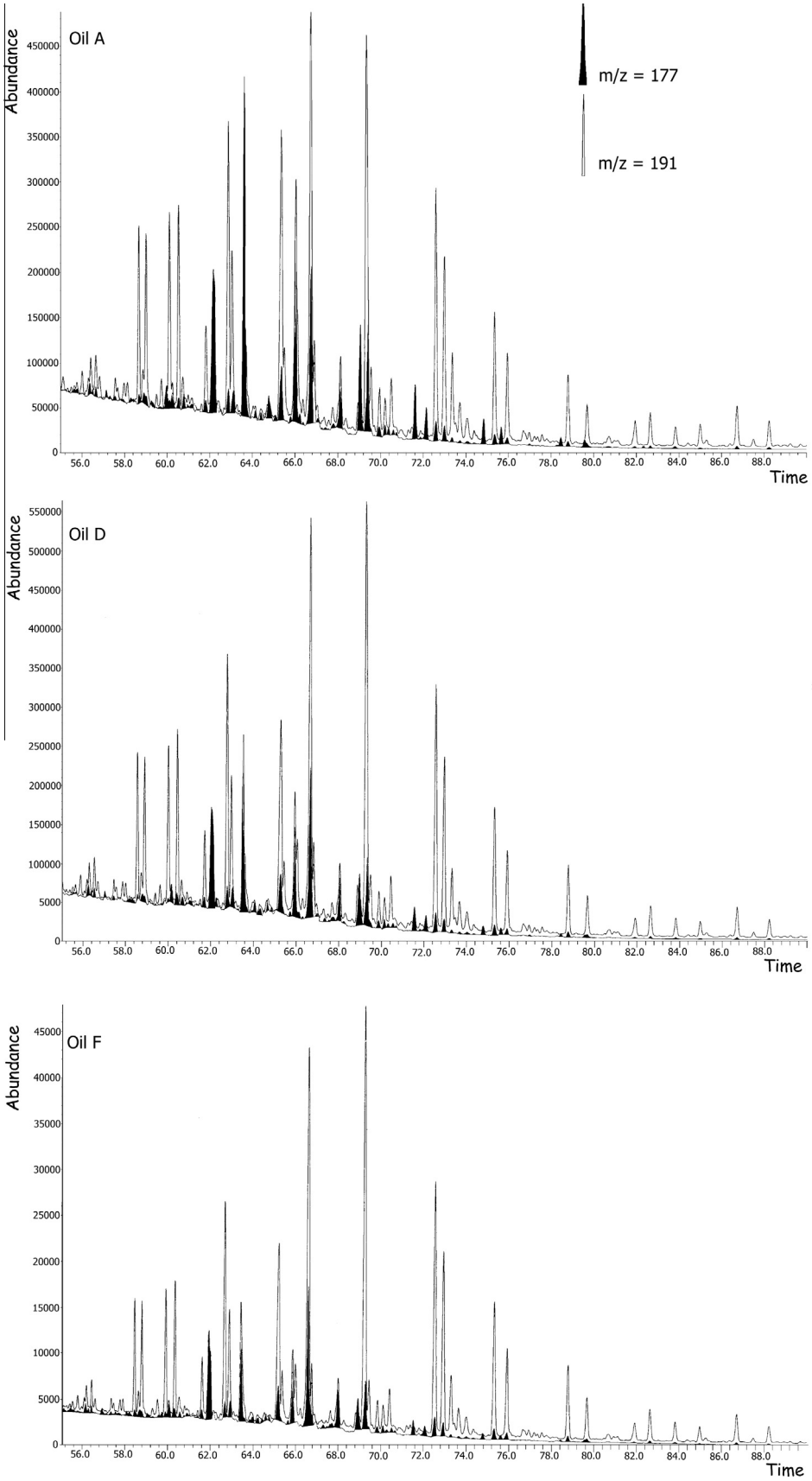

Fig. 8. *m*/*z* 191 and *m*/*z* 177 mass chromatograms showing a comparison of the distribution of hopanes in the saturated hydrocarbon fractions extracted from Oils A, D, and F. Note that in both mass chromatograms, the hopanes elute at different retention times indicating the presence of 25-norhopanes.

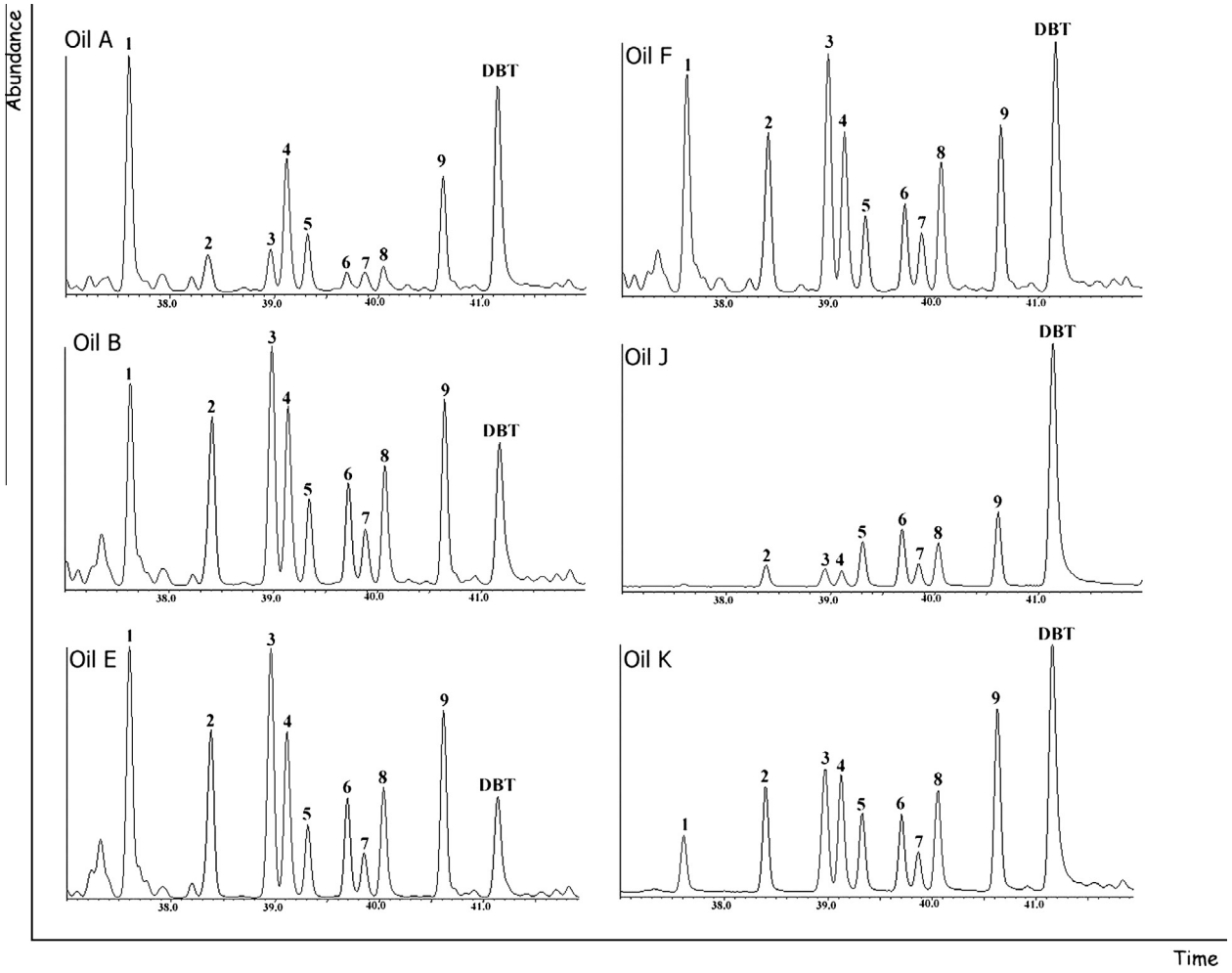

Fig. 9. Mass chromatograms of the distribution of C_4-alkylnaphthalenes and dibenzothiophenes (m/z 184) in selected oil samples. 1: 1357TeMN, 2: 1367 TeMN, 3: 1246, 1247, 1467TeMN, 4: 1257 TeMN, 5: 2367 TeMN 6: 1267 TeMN, 7: 1237 TeMN, 8: 1236 TeMN, 9: 1256, 1235 TeMN. DBT: dibenzothiophene.

resolution to better differentiate the biodegradation level due to some variations in the MS score for C_4-alkylnaphthalenes and steranes used in the calculations of MN2 values. For example, oils B and D both show biodegradation levels 4 to 5 (moderate to heavy) in the PM scale, while in the Manco scale theses oils are well differentiated, with oil D being classified as much more altered (MN2 = 716 vs. MN2 = 500 for oil B) based on the alteration of C_4-alkylnaphthalenes (MS = 3 vs. MS = 0 for oil B).

The Manco number (Larter et al., 2012) allows for a more adequate description of the level of biodegradation of heavy and extra heavy crude oils (PM level 5–8). The precise determination of the extent of biodegradation is crucial in the choice of the different exploitation strategies that may be applicable depending on the physical properties of the oils; largely the oil viscosity.

Even though the MN2 values vary over a wide range of 500–963 (Table 3), there are no significant variations in the API gravity values of the crude oils (Table 1). Larter et al. (2012) found that there is not a simple relationship between the oil viscosity and the Manco number. Thus, processes other than biodegradation can be important in controlling in-reservoir oil properties. Independently of the level of biodegradation, the initial oil composition and any secondary charge, water washing under extreme and unusual conditions, and loss of light ends from heavy oils could result in a significant increase in viscosity. In addition to biodegradation, mixing of multiple maturity oil charges could also produce variations in oil viscosity and API gravity for heavy oils.

Hence, the observed differences in MN2 values could be attributed to multiple causes. Variations in oil quality are commonly observed in biodegraded oil systems (Huang et al., 2004b; Larter et al., 2006), generally due to the coupled effect of degradation fluxes and oil charge history (Larter et al., 2003, 2006). Differences in biodegradation levels may also be caused by different communities of microorganisms or varying origins and/or maturities of the crude oils.

4.6. Final remarks

The calculated source and maturity related parameters, valid for the levels of oil biodegradation observed in this study, suggest that the crude oils investigated from the Orinoco Belt have a common origin and similar maturities. Therefore, the observed differences in the levels of biodegradation according to the PM and Manco biodegradation scales may be attributed to variations in parameters that control biodegradation rates across the study area, such as temperature, nutrient supply, proximity to oil–water contacts, or the existence varying communities of microorganisms, among other possible factors.

The levels of biodegradation of crude oils from the Orinoco Oil Belt (Junín area) were assessed using both the PM and Manco biodegradation scales. Both scales agree on the presence of different levels of biodegradation for the analyzed oils, although they are based on different groups of compounds. The PM scale uses mainly

compounds from the saturated hydrocarbon fractions (e.g., *n*-alkanes, acyclic isoprenoids, terpanes and steranes), together with aromatic steroids. On the other hand, the Manco scale considers other compounds (e.g., alkyltoluenes, naphthalene, methylnaphthalene, phenanthrene, alkyl phenanthrenes and methyldibenzothiophenes) not included in the PM biodegradation scale. Thus, a comparison between these scales allows the determination of the alteration level of both compound classes (saturated and aromatic hydrocarbons) as a result of biodegradation.

The Manco number is designed to be useful to determine the quality of heavy and extra heavy crude oils (PM level 5–8) and to adequately establish subtle differences in the extent of biodegradation that allows the implementation of appropriate exploitation strategies.

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