

**THE STUDY OF OXYGENATED COMPOUNDS FOR OIL
FINGERPRINTING APPLICATION**

Khairunisa Betariani

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for the Degree of Master of Science
The Petroleum and Petrochemical College, Chulalongkorn University
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The Study of Oxygenated Compounds for Oil Fingerprinting Application

Miss Khairunisa Betariani

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Petrochemical Technology
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การศึกษาศารประกอบไฮโดรคาร์บอนที่มีออกซิเจนเป็นองค์ประกอบสำหรับการวิเคราะห์
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การรั่วไหลของน้ำมันส่งผลกระทบต่อสภาพแวดล้อมทางทะเลและชายฝั่งเป็นอย่างมาก การระบุ
 ลักษณะของปิโตรเลียมหรือผลิตภัณฑ์จากปิโตรเลียมที่รั่วไหลในสิ่งแวดล้อมไปยังหินดินกำเนิดเป็นเครื่องมือ
 สำคัญที่ช่วยในการแก้ไขปัญหาผลกระทบต่อสิ่งแวดล้อมและความรับผิดชอบทางกฎหมาย โดยองค์ประกอบ
 และคุณสมบัติทางกายภาพของน้ำมันที่รั่วไหลอาจมีการเปลี่ยนแปลงในระหว่างกระบวนการย่อยสลายได้
 ดัชนีชี้วัดทางชีวภาพของปิโตรเลียมเป็นเครื่องมือสำหรับบ่งชี้ที่มาของน้ำมันแต่ละชนิด ซึ่งมีลักษณะเฉพาะ
 สำหรับน้ำมันแต่ละประเภท โดยทั่วไปแล้วดัชนีชี้วัดทางชีวภาพของปิโตรเลียม เช่น กลุ่มไฮโปเพน ถูก
 นำมาใช้อย่างแพร่หลายสำหรับการวิเคราะห์ลายนิ้วมือน้ำมัน โดยปฏิบัติตามวิธี **NORDTEST** นอกจาก
 กลุ่มไฮโปเพนแล้ว องค์ประกอบเฮเทอโรอะตอม (ไนโตรเจน ซัลเฟอร์ และออกซิเจน) ยังสามารถนำมา
 พิจารณาสำหรับการวิเคราะห์ลายนิ้วมือน้ำมันเพื่อเป็นข้อมูลสนับสนุนในการสืบหาแหล่งที่มาของน้ำมันที่
 รั่วไหล สารประกอบไฮโดรคาร์บอนในน้ำมันมีออกซิเจนเป็นองค์ประกอบสูงถึง 2% โดยน้ำหนัก ซึ่ง
 สามารถเกิดขึ้นได้ในหลายรูปแบบ ได้แก่ แอลกอฮอล์ ฟีนอล อีเทอร์ กรดคาร์บอกซิลิก และคีโตน
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 เป็นกรดของน้ำมันดิบ อีกทั้งยังสามารถคงอยู่ในน้ำได้นาน ในการศึกษานี้ได้ทำการศึกษาระยะยาวของ
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 น้ำมันที่รั่วไหลโดยใช้ **GC-TOFMS** อย่างไรก็ตาม ความเข้มข้นของสารประกอบไฮโดรคาร์บอนที่มี
 ออกซิเจนเป็นองค์ประกอบลดลงอย่างมีนัยสำคัญ ภายในระยะเวลา 60 วัน ภายหลังจากย่อยสลายทางชีวภาพ
 การเกิดออกซิเดชันด้วยแสง การระเหย และการละลาย การศึกษานี้แนะนำอัตราส่วนของกรดอะไซคลิกอะ
 ลิฟาติกและกรดแนฟเทนิคไซคลิก เพื่อแยกความแตกต่างของน้ำมันดิบและน้ำมันเชื้อเพลิง โดยอัตราส่วน
 ของอะไซคลิกต่อไซคลิก (A/C) ของตัวอย่างน้ำมันดิบที่ผ่านการจำลองการรั่วไหล มีค่าอยู่ระหว่าง 0.67
 ถึง 2.59 ในทางตรงกันข้าม อัตราส่วน A/C ของน้ำมันเชื้อเพลิงที่ผ่านการจำลองการรั่วไหล จะมีค่าน้อย
 กว่า 0.65 แม้ว่าการศึกษาด้วยดัชนีชี้วัดทางชีวภาพและแผนภาพความสัมพันธ์จะยังคงเป็นที่ต้องการเพื่อ
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Oil spills have a large impact on the marine and coastal environments. The characterization of petroleum or its products spilled in the environment to its source rocks is an important tool to assist in the resolution of issues of environmental impact and legal responsibility. The composition and physical properties of spilled oils have changed during the weathering process. Petroleum biomarkers is a tool for defining the origin of each oil, which is unique for each type of oil. In general, petroleum biomarkers (i.e., hopane group) are widely used for oil fingerprinting by following the NORDTEST methodology. Besides the hopane group, heteroatom-containing (N, S, and O) components can be considered for oil fingerprinting applications to provide supporting information in order to find oil spill origin. Oxygenated compounds in crude oil are up to 2% by weight. They are in various forms; alcohol, phenol, ether, carboxylic acid, and ketones. Carboxylic acid-containing compounds, naphthenic acids, are responsible for crude oil acidity and stay long in the aquatic environment. In this study, oxygenated compounds distribution is studied to provide supporting information to identify the oil spill origin using GC-TOFMS. However, the concentration of the oxygenated compound decreased significantly after 60 days of weathering due to biodegradation, photo-oxidation, evaporation, and dissolution. This study proposes the ratio of acyclic aliphatic and cyclic naphthenic acid to differentiate crude oil and fuel oil. The acyclic to cyclic (A/C) ratios of weathered crude oil samples are range from 0.67 to 2.59. In contrast, the A/C ratio of weathered fuel oil is less than 0.65. Although, biomarker analysis and correlation plots are still required to confirm the source of the oil samples.

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CHAPTER 1

INTRODUCTION

Oil and petroleum product is a part of our daily life nowadays. The stage of oil production, such as exploration, transportation, and refinery, can cause oil leaks to the environment. Accidental spills of oil and petroleum products significantly impact the marine environment. The type of petroleum product released into the environment is crude oils, condensates, heavy oils, light oils, and refined products. The oil spills have resulted in fouled coastlines, polluted fisheries, lost tourism revenue, dead and injured wildlife. Large oil spills incidents happened in the past, such as the Ixtoc 1 in 1979, the Atlantic Empress in 1979, the Persian Gulf War in 1991, the Fergana valley oil spill in 1992, and BP's Deepwater Horizon in Mexico in 2010. In Thailand, there was a large oil spill in Rayong province in 2013 because of the leakage of the pipeline operated by PTT Global Chemical Plc. The leak was the fourth major oil spill in the country's history. On 25 January 2022, there was an oil spill incident in the same province. An underwater crude oil pipeline that belongs to Star Petroleum Refining Public Company Limited (SPRC) was leaked at an offshore oil transfer point.

Some oil spill cases still happen, and forensic investigation is needed to determine the spilled source from an unknown source or origin. The identification of oil spill is an environmental forensic tool to compare source and spill samples. Thus, the oil fingerprinting technique needs to develop to identify the oil spill source. After the oil is spilled into the ocean, it has undergone the weathering processes (e.g., evaporation, photo-oxidation, dispersion, dissolution, and biodegradation). These processes significantly affect chemical fingerprints (Tarr *et al.*, 2016). Hence, the identification method for oil fingerprinting becomes an interesting and challenging topic to study.

NORDTEST method is the standard method for oil fingerprinting. This method includes identification using gas chromatography equipped with flame ionization detection (GC-FID), gas chromatography equipped with mass spectrometry (GC-MS), and specific diagnostic ratios to confirm the analysis of oil fingerprinting. GC-MS has been widely used in oil spill fingerprinting studies due to its excellent

sensitivity and availability of commercial electron ionization (EI) spectral libraries. Besides NORDTEST as the well-established procedure, new analytical developments have been applied in oil fingerprinting, such as advanced mass spectrometry. Those methods are GCxGC (comprehensive two-dimensional gas chromatography), GC-MS/MS (gas chromatography coupled to tandem mass spectrometry), GC-IRMS (gas chromatography coupled to isotopic resolution mass spectrometry), FT-ICRMS (Fourier transform ion cyclotron mass spectrometry) (Bayona *et al.*, 2015). This new analytical development may improve the diagnostic capabilities of oil fingerprinting. However, the instruments are limited in availability and expensive. Other supporting information for oil fingerprinting is needed to study to provide accessible instruments and methods.

Crude oil contains complex mixtures of hydrocarbons and non-hydrocarbon that range from volatile compounds to nonvolatile compounds. Petroleum components are commonly classified into four groups called SARA: saturates, aromatics, resin (a wide variety of compounds containing oxygen, sulfur, and nitrogen), and asphaltenes. The oxygenated compounds in petroleum are up to 2% by weight. They are in the form of alcohol, phenol, ether, carboxylic acid, ketone, and carboxylic acid ester. The oxygenated compounds have stayed long in the aquatic environment (Zan *et al.*, 2019).

In this study, the distributions of oxygenated compounds in crude oils and fuel oils, both fresh and weathered oil samples, were investigated using GC-TOFMS to provide supporting information for identifying the oil spill origin.

CHAPTER 2

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Crude Oil

Crude oil is an unrefined petroleum product composed of a highly complex mixture of hydrocarbon and nonhydrocarbons such as nitrogen, sulfur, and certain metals (e.g., nickel vanadium, and so on). Crude oils range from small, simple, volatile, and distinct compounds (e.g., methane) to extremely large, complex, nonvolatile, and colloiddally dispersed macromolecules (e.g., asphaltenes). The composition of crude oil is different depending on its source. Crude oil composition has been classified into aliphatic, aromatic, polar, and asphaltene compounds. These fractions have different chemical fingerprints (Hashemi-Nasab and Parastar, 2020).

Crude oil is typically classified to geographic location, API gravity, and sulfur content. Geographic location is commonly classified based on where the oil is recovered. American Petroleum Institute (API) gravity refers to a density scale. Crude oil is classified as heavy and light crude oil. The third-way crude oil is commonly classified is based on sulfur content. Low sulfur contained crude oil is called sweet and if it has high sulfur contained it is called sour. Sour crude oils commonly have a sulfur content of 1.0-2.0 wt%, but some have levels >4% (Demirbas *et al.*, 2015).

Remain plants and animals that lived millions of years ago were covered by sand, silt, and rock layers. After being undergone pressure and heat, it turns into crude oils (Eia, 2020). Crude oil formation is shown in Figure 2.1.

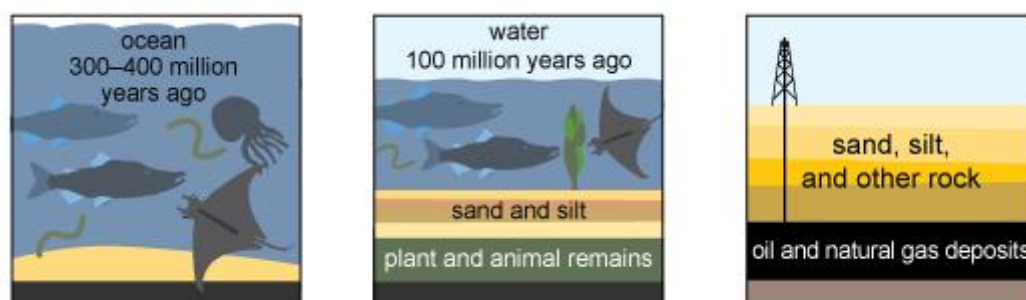


Figure 2.1 Crude oil formation diagram (Eia, 2020).

2.2 Processed Oil

Processed oils or refined products are products of crude oils that have passed processes such as distillation, cracking, treating, and reforming. In the distillation process, the larger hydrocarbons, with the high boiling points, turn back into liquids at the base of the column and the smaller hydrocarbons stay as gases. Several hydrocarbons have a low boiling point at the top of the column (between 20 °C to 70 °C). The physical and chemical characteristics of processed oil usually are different from crude oil. The distillation unit is the first step of refining. Fractional distillation is illustrated in Figure 2.2.

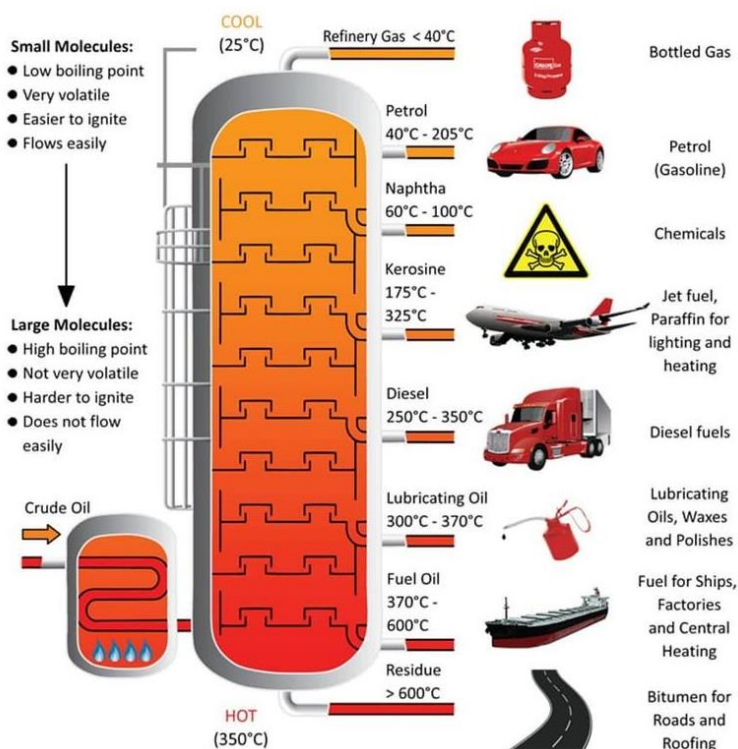


Figure 2.2 Fractional distillation of crude oil (Science-Resources, 2009).

2.3 Petroleum Chemical Fingerprinting

2.3.1 Chemical Composition of Oil

Crude oil contains complex mixtures of hydrocarbons and non-hydrocarbon from volatile compounds to nonvolatile compounds. Petroleum

component is commonly classified into four groups called SARA: saturates, aromatics, resin, and asphaltenes.

Saturates are a group of hydrocarbons consisting of carbon and hydrogen bonds with no carbon-carbon double bonds. In most crude oil, saturates are the main class of hydrocarbons that have straight-chain, branched chains (paraffin), and cycloalkanes (naphthenes). Branched cycloalkanes contained multiple condensed five- or six-carbon rings are biomarker terpanes and steranes. Smaller cyclic biomarkers, sesquiterpenes, and diamondoids can be valuable for the identification of the source of lighter petroleum products.

Alkenes (olefins) are partially unsaturated hydrocarbons consisting of one or multiple double-carbon bonds. Crude oil rarely contains olefin, but olefin has been forming during the refining process.

Aromatic hydrocarbons are cyclic hydrocarbons that contain benzene rings. Aromatic petroleum can be divided into monoaromatic hydrocarbons such as benzene, toluene, and ethylbenzene, and xylene isomer (BTEX) and other alkyl-substituted benzene compounds (c_n -benzenes) and polycyclic aromatic hydrocarbons (PAHs), e.g., C_0 - to C_4 -naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene homologous.

Resin compounds have heterocyclic hydrocarbons and contain other organic materials such as (nitrogen, oxygen, and sulfur-containing PAHs), phenols, acids, alcohols, and monoaromatic steroids. These compounds are the most soluble in the polar solvent because of their polarity. Sulfur compounds can be present in several forms: hydrogen sulfide, elemental sulfur, thiophenes, mercaptans, benzothiophenes, and naphthobenzothiophenes. In general, organic nitrogen is present in crude oils as alkylated aromatic heterocycles with more neutral pyrrole and carbazole structures than basic pyridine and quinoline forms. Hydrocarbons react with oxygen to form various oxygen-containing compounds, such as phenols, cresols, and benzofurans. The concentration of nitrogen- and oxygen-containing compounds are commonly less than PAHs in crude oil, ranging from 0.1%-3%. However, the boiling point interval of the crude oil fraction commonly will increase the oxygen content in the crude oil fraction.

Asphaltenes are a class of very large heteroatom-containing compounds that are dispersed as colloids in petroleum. Table 2.1. shows the composition of several oils and petroleum products (Wang *et al.*, 2016).

Table 2.1 The composition of crude oils and petroleum products

Group	Compound Class	Gasoline	Diesel	Light crude	Heavy crude	IFO	Bunker C
Saturated		50-60	65-95	55-90	25-80	25-45	2040
	Alkanes	45-55	35-45				
	Cyclo-alkanes	~5	30-50				
	waxes		0-1	0-20	0-10	2-10	5-15
Olefins		5-10	0-10	-	-	-	-
Aromatics		25-40	5-25	10-35	15-40	40-60	30-50
	BTEX	15-35	0.5-2	0.1-2.5	0.01-2	0.05-1	0-1
	PAHs		0.5-5	0.5-3	1-4	1-10	1-10
Polar compounds		-	0-2	1-15	5-40	15-25	10-30
	Resins	-	0-2	0-10	2-25	10-15	10-20
	Asphaltenes	-	-	0-10	0-20	5-10	5-20
Sulphur		<0.05	0.05-0.5	0-2	0-5	0.5-2	2-4
Metals (ppm)				30-50	100-500	100-1000	100-2000

BTEX = benzene, toluene, ethylbenzene, and xylenes; PAHs = polycyclic aromatic hydrocarbons.

2.3.2 Petroleum Biomarkers

Biomarkers are complex molecules that are derived from formerly living organisms. They have been used by petroleum and reservoir geochemists to characterize the thermal maturity of the oil, evaluate migration and degree of biodegradation, determine depositional environmental conditions, and determine the

age of the source rock for petroleum. Nowadays, biomarkers have become important in forensic investigations of oil spills because they can keep most of the original carbon structure of oil and resist the weathering process than other hydrocarbon groups in crude oil. Moreover, biomarkers are formed under various geological conditions and ages, which might show the different fingerprints. Thus, analysis of biomarkers in oil provides important information for determining oil spill sources (Wang *et al.*, 2016).

The basic structure of biomarker compounds in petroleum is isoprene, consisting of isoprene subunits called terpenoids or isoprenoids. In addition, the terpenoids are classified based on the approximate number of isoprene subunits, as shown in Table 2.2.

Table 2.2 Families of terpenoids (Wang *et al.*, 2007)

Hemiterpane (C ₅)	Containing one isoprene subunit
Monoterpanes (C ₁₀)	Containing two isoprene subunit
Sesquiterpanes (C ₁₅)	Containing three isoprene subunit
Diterpanes (C ₂₀)	Containing four isoprene subunit
Sesterterpanes (C ₂₅)	Containing five isoprene subunit
Triterpanes and steranes (C ₃₀)	Containing six isoprene subunit
Tetraterpanes (C ₄₀)	Containing eight isoprene subunit
Polyterpanes (C _{5n (n>8)})	Containing nine or more isoprene subunit

2.3.2.1 Acyclic or Aliphatic Biomarker

A large number of aliphatic isoprenoids hydrocarbon is detected in oils, coals, shale, and dispersed organic materials. The linkages between isoprene subunits might be regular (head-to-tail) or irregular (head-to-head or tail-tail) linkages. Phytane (C₂₀H₄₂) is typical of a regular, acyclic isoprenoid with four head-to-tail linked isoprenoid units. Phytane is the most common abundant isoprenoid in oil and has been generally used to estimate the degree of oil biodegradation in the environment. Examples of irregular isoprenoids are squalene (C₃₀H₆₂) and botryococcane (C₃₄H₇₀). Figure 2.3. shows the example of acyclic biomarker structure.

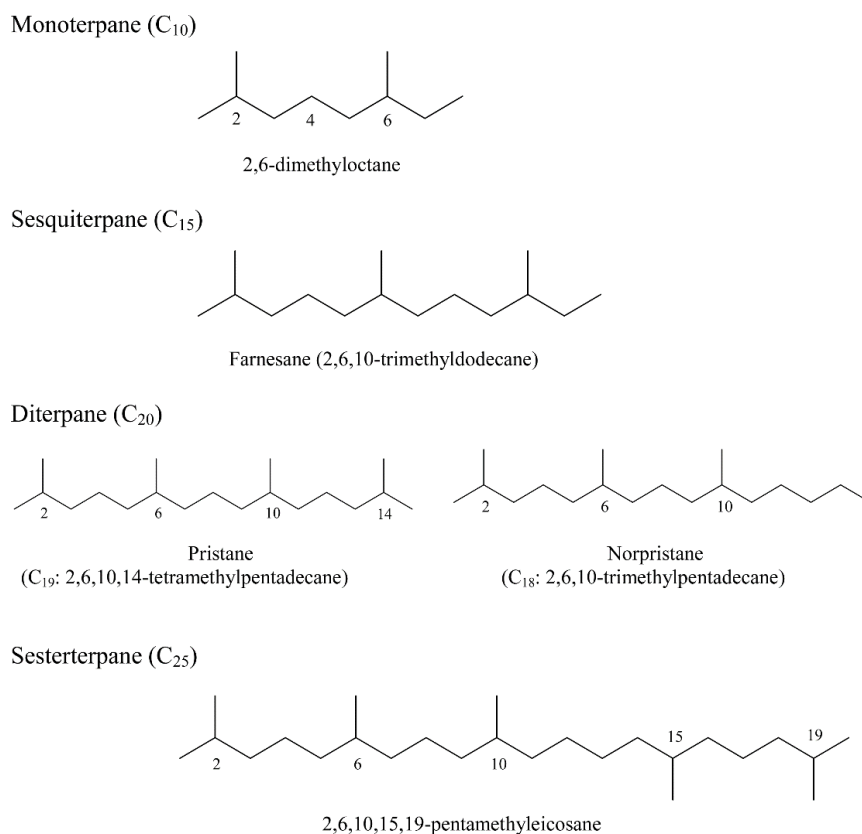


Figure 2.3 Molecular structures of representative acyclic terpenoid compounds (Wang et al., 2016).

2.3.2.2 Cyclic Biomarker

Oil's most common cyclic biomarkers are terpanes, steranes (irregular cyclic terpenoid compounds), and aromatic steranes. Terpenes, including bicycle, tricyclic, tetracyclic, and pentacyclic, are found in crude oil. An example of pentacyclic triterpenes is hopanes which contain 27-35 carbon atoms. The large abundance and thermodynamic stability of hopanes with the 17 α (H), 21 β -configuration with C₂₇ to C₃₅ are more commonly used to characterize petroleum than other empirics ($\beta\beta$ and $\alpha\beta$) series.

Steranes contain 21-30 carbons that include regular steranes, rearranged diasteranes, and mono- and tri- aromatic steranes. The most common steranes are the regular C₂₇-C₂₈-C₂₉ homologous sterane series (cholestane, ergostane, and stigmastane). These series are helpful for chemical fingerprinting because of their

high source specificity (Wang *et al.*, 2016). Figure 2.4. shows examples of molecular structures of cyclic biomarker compounds in oil, and Figure 2.5 shows the range of carbon atoms of cyclic biomarkers in oil.

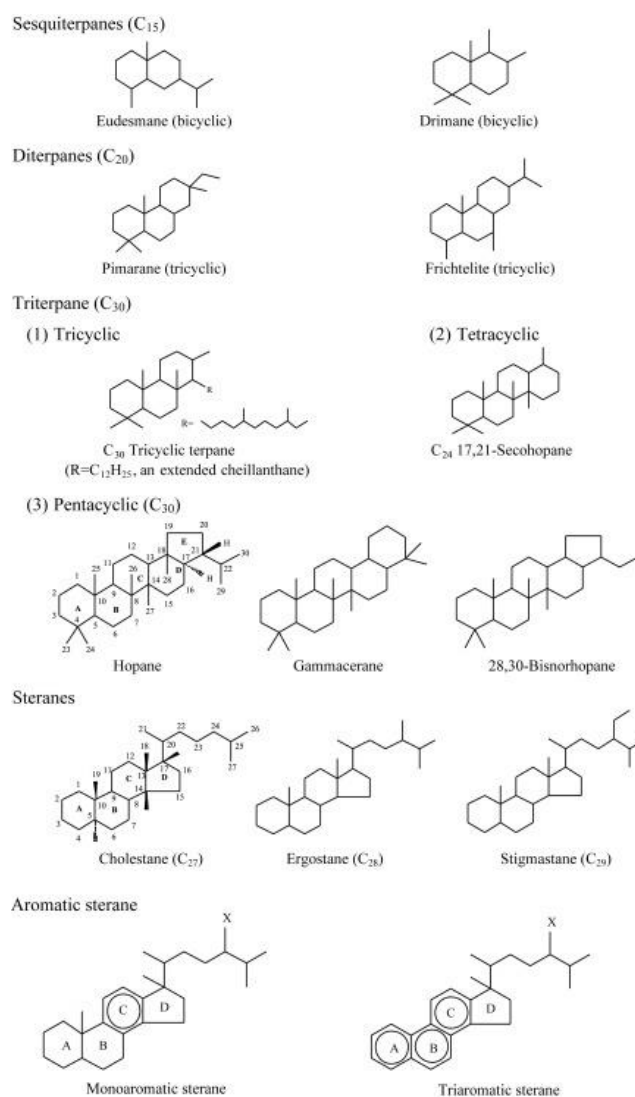


Figure 2.4 Molecular structures of example cyclic biomarker in oil.

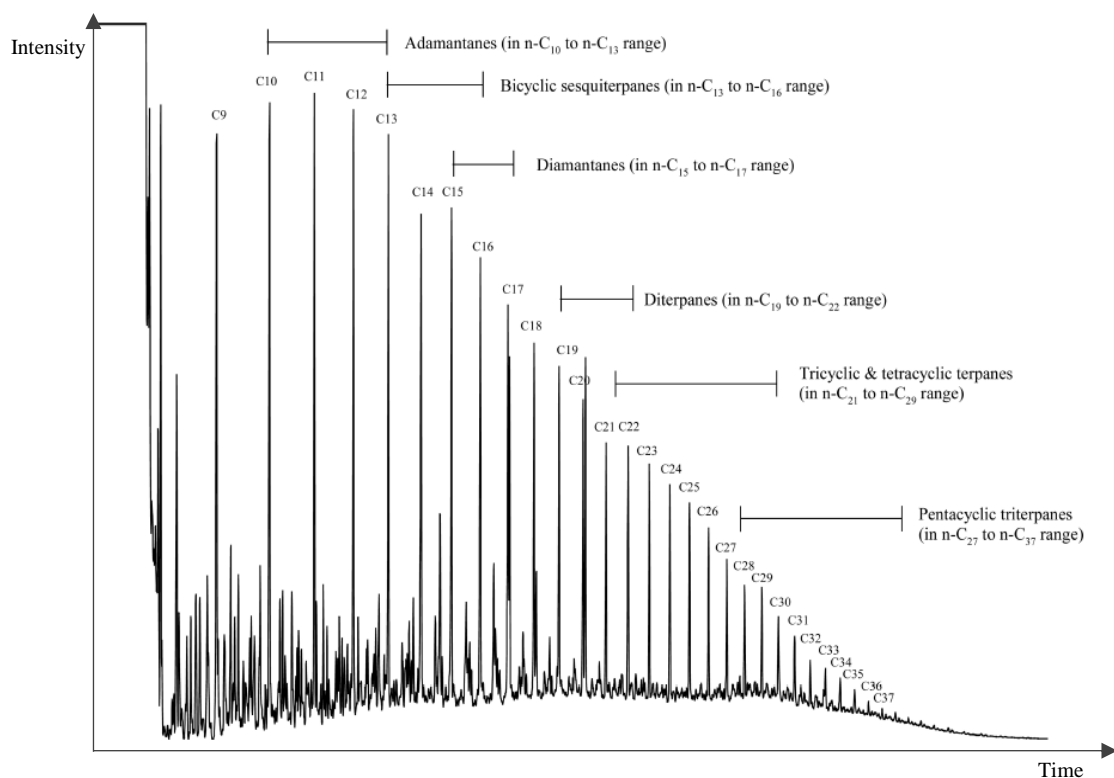


Figure 2.5 Range of carbon atom of cyclic biomarkers in crude oils and processed oils.

2.3.3 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds, that primarily have colorless, white, or pale-yellow solids. They are an omnipresent group of several hundred related compounds. They have carcinogenic and mutagenic effects. PAHs are a byproduct of incomplete combustion during the formation of crude oil. Chemically the PAHs contain two or more benzene rings bonded in the linear, cluster, or angular arrangement. Figure 2.6. shows the molecular arrangement of PAHs. PAHs that contain up to six aromatic rings are classified as “small” PAHs while more than six aromatic rings are classified as “large” PAHs. The general properties of PAHs are high boiling points, low vapor pressure, and low solubility in water (Wang *et al.*, 2016).

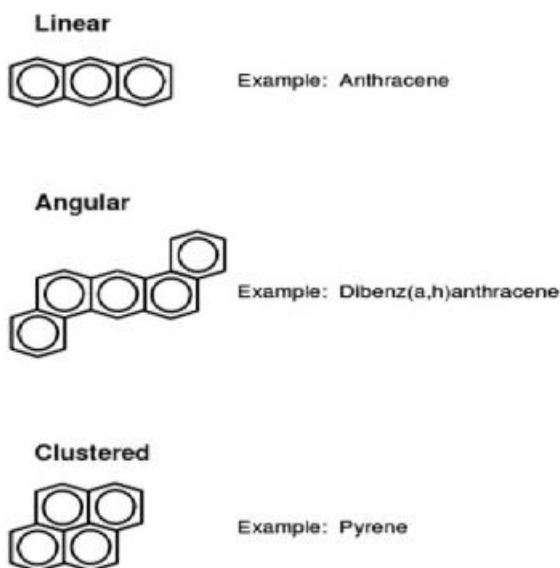


Figure 2.6 Molecular Arrangement of PAHs.

The primary source of PAHs can be divided into three types (pyrogenic, petrogenic, and biological). Pyrogenic PAHs are formed under high temperatures and low oxygen. Moreover, an example of the pyrogenic process is the thermal cracking of crude oil or distillation of coal. The temperature of this process is around 350 °C to more than 1200 °C. In contrast, petrogenic is formed under low-temperature conditions during the crude oil maturation process. These petrogenic PAHs are commonly found in petroleum products. The formation temperature is vital information to provide PAHs sources identification. For example, PAHs with fewer alkylate chains might be produced under high formation temperatures.

2.3.4 Polar Compound

Polar compounds have different positive and negative charge regions because of the presence of chemical bonds with atoms such as nitrogen, oxygen, or sulfur. Heavy oils generally contain a more significant proportion of higher boiling, aromatic, and heteroatom-containing (N, S, O, and metal-containing) component. The polar heteroatom cyclic compounds are commonly contained in low concentrations. They are generally bio-refractory and persistent in the environment and they become concentrated on weathering. Many individual alkyls are homologous of N-, O-, S-containing compounds, including alkylated carbazole, quinoline, pyridine, thiophene, and dibenzothiophene have been identified in many oils. Resin is the smaller polar

compound. The asphaltenes are a large polar compound because they often make up the most significant percentage of asphalt.

2.3.4.1 Oxygenated Compounds

The oxygenated compounds in crude oil are commonly less than 2% weight. They can form in various functional groups such as alcohols, ethers, carboxylic acids, phenolic compounds, ketones, and esters. These compounds cause the acidic of crude oil (Fahim *et al.*, 2010). One of the oxygenated compounds in crude oil is naphthenic acids (NAs). Naphthenic acids contain the carboxylic acid group. They can be present as a by-product of incomplete biodegradation or thermal degradation of petroleum products. They are viscous liquids with unique odors because of the presence of phenolic and sulfur impurities. NAs are referred collectively to as a family of cycloaliphatic carboxylic acids. They have an empirical formula of $C_nH_{2n+z}O_2$, where n is the number of carbon and z is zero or negative, or integer represents the hydrogen deficiency (unsaturated degree) of NA molecule (Ajaero *et al.*, 2016). This formula can not use to describe acids that contain more than one carboxylic group and nitrogen and sulfur-containing organic acids, that also classified as NAs. Figure 2.7. shows the example of NAs with different z numbers. NAs that has only one carboxylic group (NAs) are called acyclic. The branched-chain structure is more common than the straight chain structure. The carboxylic group in NAs is generally attached to a side chain instead of cycloaliphatic ring.

NAs have boiling point between 250 and 350 °C. They have low volatility. The acidity of NAs is higher than phenol and cresylic acid but weaker than low molecular weight carboxylic acids. For the solubility in water, alkanes, alkenes, and polycyclic aromatic hydrocarbons have lower solubility than NAs. Moreover, alkaline pH promotes solubility, and the solubility is more influenced by pH than temperature. On the other hand, NAs are soluble in organic solvents and oils. Table 2.3 shows the molecular weight, density, and pKa of some commonly used model NAs. (Wu *et al.*, 2019).

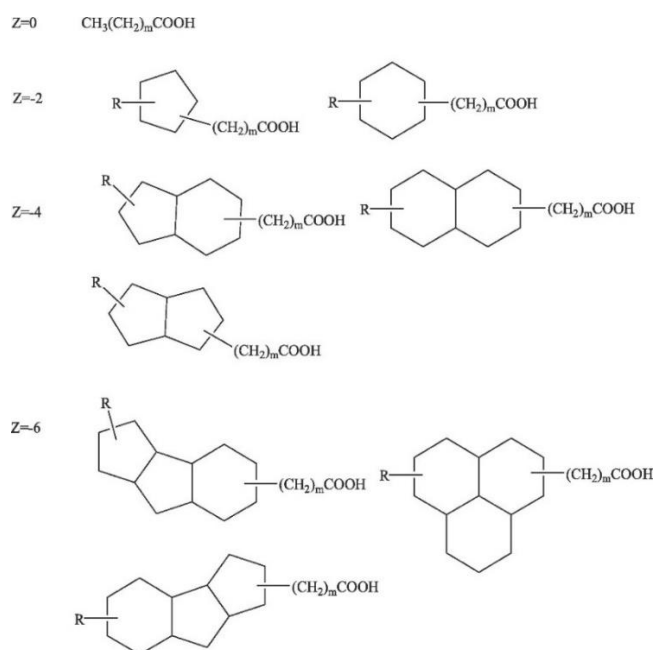
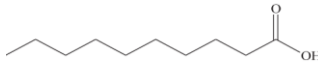
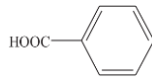
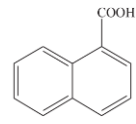
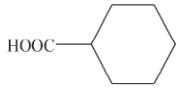
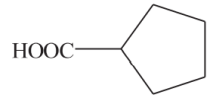
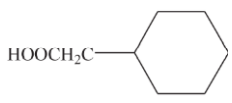


Figure 2.7 Example of NAs with different z numbers (Ajaero et al., 2016).

Note: R is an alkyl chain and Z is the hydrogen deficiency.

Table 2.3 MW, density, and pKa of model NAs (Ajaero *et al.*, 2016)

Model NAs	Structure	MW	Density(g/cm ³)/ Temperature	pKa/ 25 °C
Decanoic Acid		172.27	0.8858/40 °C	4.9
Benzoic Acid		122.12	1.320/25 °C	4.2
1-naphthoic acid		172.18	1.398/25 °C	3.64
Cyclohexane Carboxylic Acid		128.17	1.0334/22 °C	4.88
Cyclopentane Carboxylic Acid		114.14	1.053/20 °C	4.99
Cyclohexane acetic acid		142.20	1.0423/18 °C	4.80

The naphthenic acids have to separate from their parent sources for analysis, either solid- or liquid-phase extraction (Headley *et al.*, 2007; Monaghan *et al.*, 2021; West *et al.*, 2014). NAs characterization has been reported with a wide range of analysis techniques. The forensic evaluation of NAs in crude oil provides baseline information source of NAs and their composition. Fourier transform infrared spectroscopy (FTIR) and gas chromatographic flame ionization detection (GC-FID) or GC-MS methods were used in earlier studies on the identification of naphthenic acids (Ajaero *et al.*, 2016). High-molecular-weight acids cannot be resolved well with GC because of their polarity and low volatility. However, the derivative of the sample is needed to aid the molecular ion determination. There are several chemical reagents to carry out derivatization, such as urea, BF_3 /methanol, methanol/HCL, *N,N*-dimethylformamide, diazomethane, *N,O*-Bis(trimethylsilyl)trifluoroacetamide, *N*-methyl-*N*-(tertbutyldimethylsilyl)trifluoroacetamide, 2-nitrophenylhydrazine, and tert-butyltrimethylsilylchlorid (Monaghan *et al.*, 2021). Silylation is the common method to derivatize acid. The example of the silylation agent is BSTFA (*N,O*-Bis(trimethylsilyl)trifluoroacetamide) and MSTFA (*N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide). This derivatize method produces trimethylsilyl (TMS) esters from the carboxylic group. However, the silylation method also can derivatize hydroxyl and amino groups (Hepnet, 2020).

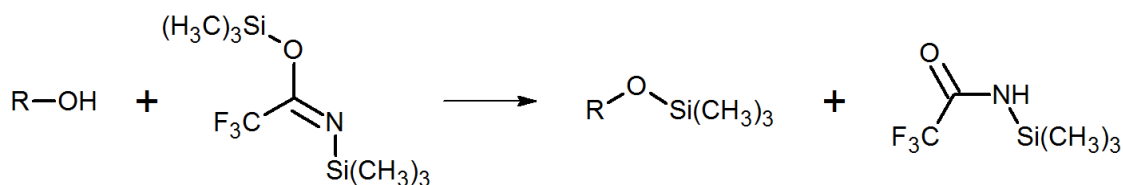


Figure 2.8. The mechanism of silylation with BSTFA (Hepnet, 2020).

2.3.5 Oil Weathering Process

After the oil is spilled into the environment, it immediately begins to undergo various physical, chemical, and biological processes that transform the oil composition. Oil spreads to form a thin layer called an oil slick on the sea surface.

The oil slicks undergo several natural processes together to degrade the oil slick. These processes are called oil weathering processes. These processes are complex, self-completing, and act simultaneously (Mishra and Kumar, 2015). Figure 2.9. shows the oil weathering process, including spreading, evaporation, dispersion, dissolution, oxidation, and biodegradation. The speed of these processes depends on weathering conditions (location, wind, wave, and sunlight) and oil properties such as API gravity, volatility, and viscosity. Furthermore, the most significant effect at the early stage of the oil spill is spreading, evaporation, dispersion, emulsification, and dissolution. Oxidation, sedimentation, and biodegradation have the most significant effect at the later stage of a spill. The later stage is the longer-term process that will determine the fate of the oil spilled (Itopf, 2014). Figure 2.10. shows the fate of typical oil. The descriptions of weathering processes are described in this following section.

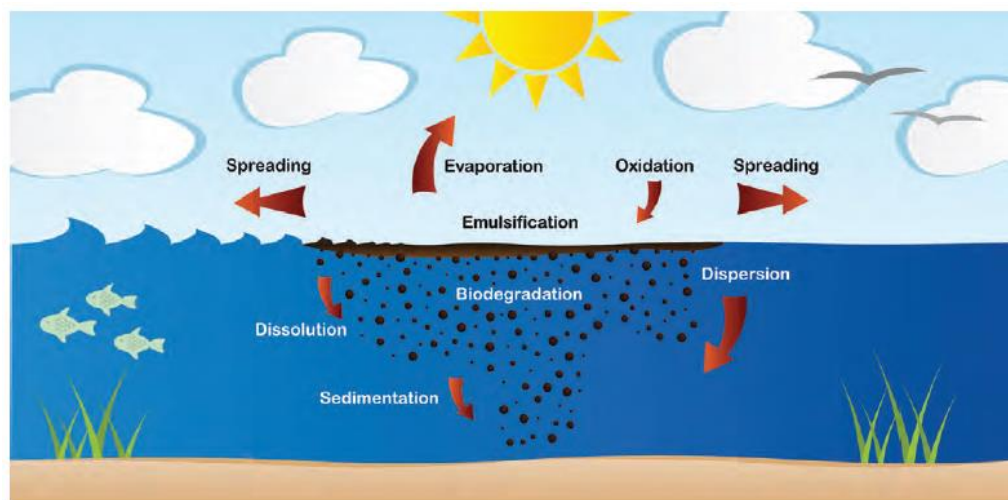


Figure 2.9. Overview of the major oil weathering processes (Itopf, 2014).

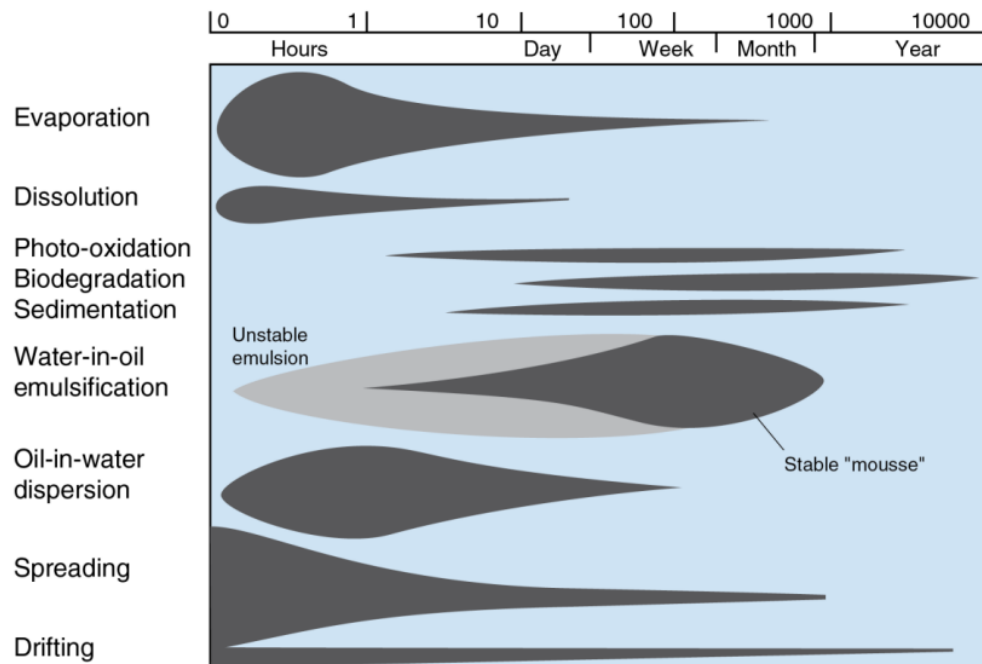


Figure 2.10 The estimation time of each weathering process (Hellström, 2021).

2.3.5.1 Spreading

Oil starts to spread over the seawater immediately after it is spilled. The speed of spreading is affected by the viscosity of oil, and the volume spilled. Moreover, it is also affected by waves, turbulence, tidal stream, and currents. The oil initially spreads as a coherent slick, but after a few hours, the slick generally begins to break up because of wind and wind and will tend to form narrow bands.

2.3.5.2 Evaporation

Light molecular weight component in oil is evaporated into the atmosphere. The rate of evaporation is affected by wind speed, ambient temperatures, and the volatility of oil. For example, the oil with a higher fraction of light component or low boiling point component with a boiling point of less than 200 °C will evaporate within 24 hours. The rate of evaporation also depends on the initial spreading rate. For instance, a large surface area has a higher evaporation rate. Moreover, warm temperatures, high wind speeds, and rough seas are increasing the evaporation rate. Thus, the spill of light oil products such as kerosene and gasoline might evaporate entirely within a few hours.

2.3.5.3 *Dispersion*

The wave and turbulence of the sea are caused the slick to break into a droplet. The droplet becomes spread into the upper layer of seawater. The rate of dispersion mainly depends upon the characteristic of oil and sea state. Moreover, dispersion occurs very quickly for light molecular weight oil.

2.3.5.4 *Emulsification*

The oil spill is mixed with water and form water-in-oil emulsions. This process leads to thickening oil and increases the volume of pollutants by three to four times. Although, emulsification can reduce other natural weathering processes and the persistence of light and medium crude oils on the sea surface.

2.3.5.5 *Dissolution*

Dissolution of oil takes place at the surface between oil and seawater. The dissolution rate is affected by the oil composition, surface area of oil, spreading, water temperature, and degree of dispersion. The heavy components in oil are almost insoluble in the sea, and the light components are slightly soluble. Light components usually are lost by evaporation. Although, the concentration of dissolved oil in seawater is commonly not more than 1 ppm.

2.3.5.6 *Photo-oxidation*

Photo-oxidation is a process that oil reacts with oxygen which is promoted by sunlight. This process leads to the formation of soluble products or persistent compounds called tars. The rate of this process is very slow, even in strong sunlight. In general, thin oil film breaks down less than 0.1% per day and tends to oxidize from the thin layer to oil residue rather than degrade. A typical example of this process is tarballs. Thick layers are formed to protect the softer part by oxidation.

2.3.5.7 *Sedimentation and Sinking*

Oil droplets interact with solid particles and organic matter suspended in the sea, and the droplet will dense and slowly sink to the seabed. This process is called sedimentation. Furthermore, sedimentation is one of the long-term processes that lead to the accumulation of spilled oil in the sea. However, the sinking of bulk oil is observed only in shallow water close to the shore.

2.3.5.8 Biodegradation

Biodegradation is when the micro-organism in the sea, including bacterial, yeast, fungi, unicellular algae, and protozoa, changes the oil component into the energy source. Biodegradation is a long-term mechanism for the removal of a trace of oil in shorelines. The rate and extent of biodegradation are affected by the oil properties, the availability of oxygen, nutrients, and temperature. However, the variety of factors that affect biodegradation makes it challenging to predict.

2.3.6 Weathering Effect on Chemical Fingerprinting

Zhang et al. 2015 studied on weathering characteristics of crude oils from the Dalian oil spill by using GC-MS. After going under the simulated weathering process for 95 days, the distribution of aliphatic hydrocarbon with low molecular weight, e.g., normal alkane and isoalkane ($< n-C_{13}$), is affected by the weathering process and it will increase the unresolved complex mixture. However, some aliphatic compound such as pristane (Pr) and Phytane (Ph) is still found and shows the strong capability of weathering resistance and becoming dominant peak. Furthermore, the ratios of $n-C_{17}/Pr$, $n-C_{18}/Ph$, and Pr/Ph have indicated oil biodegradation. The Pr/Ph ratio decreasing can indicate Ph is more resistant than Pr. Biomarker distribution of terpane and sterane indicated it might be unchanged by the weathering process. Thus, the biomarker compounds can be used as fingerprinting for oil origin identification. Ho et al. 2015 studied five classes of PAHs such as naphthalenes (N), phenanthrenes (P), dibenzothiophenes (D), fluorene (F), chrysenes (C), and their alkylated homologs. Thus, chrysenes (C) are the most resistant to weathering among PAHs (Ho *et al.*, 2015; Mishra and Kumar, 2015).

Zan *et al.*, 2019 studied on variation and distribution of naphthenic acid in Dalian Bay oil spill sediment. Dalian Bay oil spill accident happened in 2010. This experiment used a GC-MS to quantify NAs in the sediment sample. The detector, which is a mass spectrometer, was operated in full scan mode with electron impact source, and mass ion scanned from $m/z = 55-550$ with 1 scan/s. Generally, acyclic acids can be degraded more quickly than cyclic compounds in the environment. From this research, the highest abundance of NAs was recorded in 2010 when the oil spill

happened. Afterward, the concentration of NAs decreased and stayed at relatively stable levels. NAs were 10-30 times higher than total PAHs in the sediment of Dalian Bay. However, they can be removed through biodegradation and natural mineralization in the sediment surface.

2.4 Oil Spill Identification Methodology

2.4.1 Chemical Fingerprinting Techniques

Oil fingerprinting is commonly used to determine the source of an oil spill by matching compositional parameters of the potential source and the spilled oil. Nowadays, there is various analytical fingerprinting technique of oil such as gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), high-performance chromatography (HPLC), thin-layer chromatography (TLC), ultraviolet (UV) spectroscopy and spectroscopic method, and so on (Oforka, 2012). The most widely used methodology is gas chromatography that followed CEN TR15522-2 and the ASTM5739-00 standard norms. Other methodologies are development techniques that can support GC-technique to get a better result (Bayona *et al.*, 2015).

Chromatography is a separation technique of a mixture compound into their individual component based on their volatiles. There are many types of chromatography, such as liquid chromatography (LG), gas chromatography (GC), ion-exchange chromatography. Gas chromatography (GC) is the most important for oil fingerprinting because GC can show both qualitative (identify component) and quantitative (measuring amount of sample component).

Gas chromatography has three main parts. They are injector, column, and detector. The sample solution is injected into the instrument by the injector, and it will be vaporized into the vapor phase. Then, the sample stream is carried to the column by a carrier gas such as Helium or Nitrogen to separate the sample into the various component. Afterwards, the separated components are detected and measured by the detector (Morsi, 2012). Figure 2.11. shows a typical GC instrument.

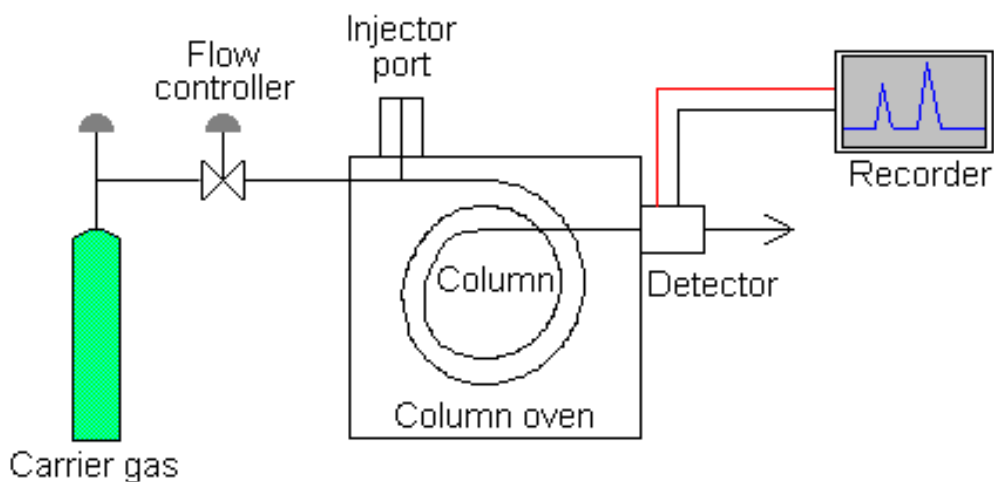


Figure 2.11 Typical Gas chromatography (GC) instrument (Morsi, 2012).

The column is the most important in this system. The separation of the various component occurs in the column. There is stationary phase and packed material in the GC column, which influences the separation of the compound and the time of separation (retention time). The column can be divided into two types, including capillary column and packed column. In general, a capillary column has a better resolution and gives better fine structure chromatography. The capillary column is widely used in environmental forensic than the packed column. The column is placed in an oven. The temperature can be controlled accurately over a wide range of temperatures. Figure 2.12 and Figure 2.13 show the picture of the capillary column and packed column, respectively.

There are many types of detectors for GC. The detector selection is based on its application. Mass spectroscopy (MS) is widely used in GC techniques because it can provide qualitative identification of various compounds and quantitative analysis. When GC is linked with mass spectrometry, electron ionization (EI) or chemical ionization (CI) are ionized compounds that elute from the GC column into charge fragments. After that, charge fragments are accelerated into the mass analyzer. The difference in mass to charge ratio will create a different signal. Moreover, the mass analyzer can detect the compound that produces ions within the mass range. Although, one-dimensional gas chromatography is limited on complex samples because it has co-elute interference that might obstruct GC information.

Thus, comprehensive gas chromatography becomes a new technique for oil fingerprinting because it has the high-resolution capacity, selectivity, and good resolution capability of co-eluted biomarkers (Bayona *et al.*, 2015; Piantanida and Barron, 2014).



Figure 2.12 Capillary column of gas chromatography (Piantanida and Barron, 2014).



Figure 2.13 Packed column of gas chromatography (Piantanida and Barron, 2014).

2.4.1.1 GC x GC-TOFMS

In the past few years, two-dimensional gas chromatography (GCxGC) has been used for source oil identification. The advantage of TOF (time of flight) is the ability to confirm oil fingerprinting and resolve co-eluted PAHs and biomarkers in oil (Bayona *et al.*, 2015). GCxGC is arranged into 2 columns. The first column is a non-polar column, and the second column is a polar column. However, some researchers are claimed that the opposite arrangement can provide a better result of non-polar components (Bayona *et al.*, 2015). Figure 2.13 shows the simple diagram of the GCxGC-TOFMS instrument. The thermal modulator placed between two columns is important in this instrument. It ensures that all streams from the first column reach the temperature before it releases into the second column (Leco, 2019)

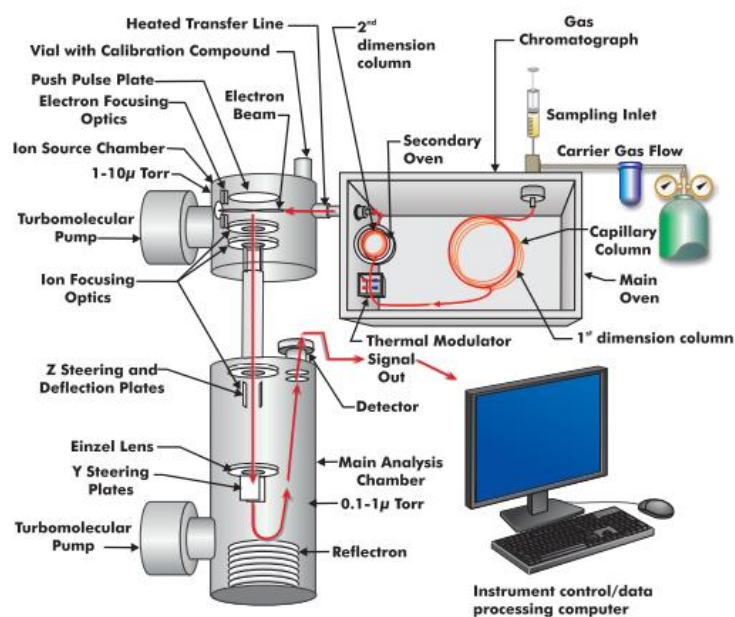


Figure 2.14 Diagram of GCxGC-TOFMS (Leco, 2019).

2.4.2 NORDTEST Methodology for Oil Spill Identification

NORDTEST oil spill identification system was formed in 1991. This method is a principal for oil spill identification in Scandinavian countries and other European countries. The NORDTEST method has three steps procedure. The first step is screening all of the sample characterizations by GC-FID. The second step is to

collect data using GC-MS and compare it with the previous step's data. The final step is to identify the possible differences. The conclusion would be either identified or non-identity (Hansen *et al.*, 2002).

This methodology has been used for oil spill identification since 1991, but NORDTEST was found “Revision of the NORDTEST Methodology for Oil Spill Identification” in April 2000. This project results from collaboration with researchers from many countries, including Finland, Norway, Sweden, Denmark, and United States. The objectives of this new NORDTEST project are (1) review and assessment of public literature and (2) an update of the existing NORDTEST method into a technically more robust and legally defensible. The result of improvement Nordtest methodology was still based on the same procedure; screening GC/FID, GC/MS fingerprinting, and analytical data. The procedure chart for NORDTEST methodology is illustrated in Figure 2.15, and an analysis of oil spill identification will be performed with this following step (Nordtest, 1991).

Level 1 – GC-FID screening: prepared sample is screened by GC-FID. GC-FID with full scan mode can be used if GC-FID is not available. GC-FID provides information both qualitative and quantitative. GC-FID chromatograms give information of the overall boiling range, dominance peak of n-alkanes and isoprenoid, and weathering effect on the oil spill. Suppose GC-FID chromatogram between the oil spill and potential source is different in n-alkane distribution. In that case, the unresolved complex mixture (UCM) shapes or diagnostic ratios of n-alkane and isoprenoid without weathering effect. However, it needs to be considered in the next step to confirm the result.

Level 2 - GC/MS fingerprinting: GC/MS in the selected ion monitoring mode (GC/MS-SIM) is used for analyzing the potential source of the sample and spilled oil sample that has not been taken out from level 1. Furthermore, the distribution of oils and the diagnostic ratio of biomarkers and PAHs are determined in this step.

Level 3 - Evaluating data and weathering check: The results from level 1 and level 2 are evaluated diagnostic ratio and eliminated candidate sources that show possible differences without weathering effect. Moreover, the result from two levels is used to determine the analytical relative standard deviation (RSD). Then, the



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diagnostic ratios that are higher (i.e., RSD>95%, precisely measured and weathering resistant) are selected by using the “Student’s t” statistic tool. After that, the results are shown in a simple x-y plot, and linear regression is performed. The conclusion is based on the “match” between spill and candidate source samples.

The overall conclusion can be classified into four categories: positive match, probable match, inconclusive, and non-match. This category depends on the degree of the differences between the spill and the candidate source sample.

Positive match: The patterns of chromatograms of the spill and suspected source samples are virtually identical. The only observed differences are caused by acceptable analytical variance and/or weathering.

Probable match: The patterns of chromatograms of the spill and suspected source samples are similar except for the difference, which causes by weathering processes (e.g., loss of lower molecular weight peaks, wax redistribution, etc.) or contaminants.

Inconclusive: The patterns of chromatograms of the spill and suspected source samples are quite similar, except for the difference that is impossible to ascertain. These differences may be caused by heterogeneities of the oil quality either within the spill sample or the suspect source.

Non-match: The patterns of chromatograms of the spill samples are unlike the suspected source sample (Faksness *et al.*, 2002).

The conclusion categories should be used correlation plot analysis. For the correlation plot analysis, samples especially spilled samples need to be triplicate analyzed to calculate the analytical standard deviation of the diagnostic ratio.

Observation’s diagnostic ratio can be calculated by equation (2.1)

$$\text{Diagnostic ratio} = \frac{100 \times A}{(A + B)} \quad (2.1)$$

A and B are concentrations got from a multi-point calibration curve for biomarker compounds with the standard.

The values for A and B for biomarkers with no corresponding standard were (area of analyst) / (area of internal standard).

The accuracy of correlation analysis (equation 2.2) and diagnostic ratio calculation relies on the triplicate analysis measurement. The result can be calculated

as the relative variation 95% confidence interval or confident limit (CL) using the “Student’s t” statistic tool.

Correlation analysis of Diagnostic ratio (2.2)

$$\mu = x \pm \frac{t \times s}{\sqrt{N}} \quad (2.2)$$

Where x is mean value, the center of the distribution.

s is standard deviation.

t is student’s t distribution.

N is the number of observations.

t value is a relation between the degree of freedom (N-1) and confident level that shows in Table 2.4.

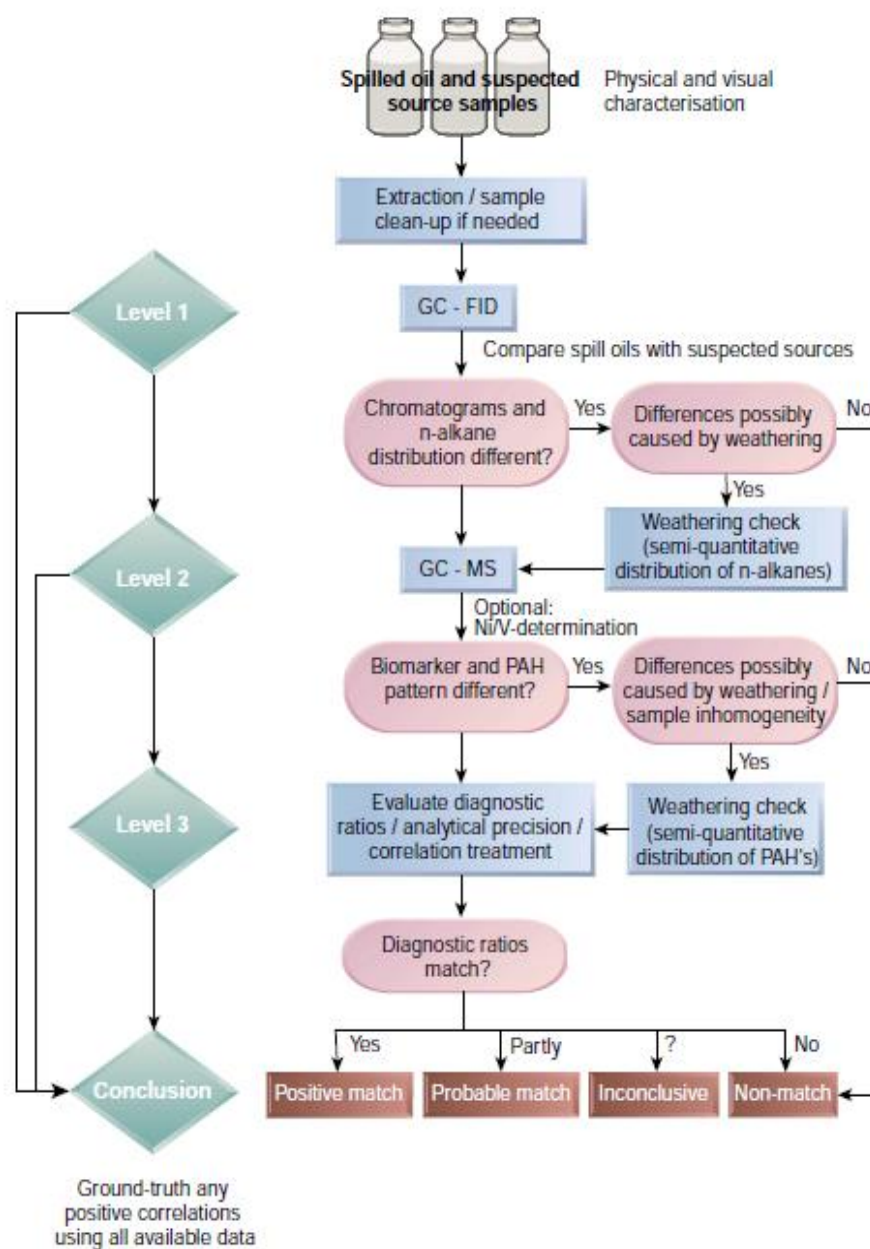


Figure 2.15 The procedure chart for NORDTEST methodology (Faksness et al., 2002).

Table 2.4 Values of Student's t (Harris, 1995)

Degree of freedom (N-1)	Confidence level (%)		
	95	98	99
1	12.706	31.821	63.657
2	4.303	6.965	9.925
3	3.182	4.541	5.841
4	2.776	3.747	4.604
5	2.571	3.365	4.032
∞	1.960	2.236	2.576

For the confidence level, 95 % confidence interval is suggested for a definition of a positive match. For example, Round Robin studied is a collaboration of 12 different laboratories from 10 countries to analyze oil spills and demonstrate the strong capability of the NORDTEST method). These examples are shown in Figures 2.16 to 2.21. Furthermore, the recommended criteria for correlation analysis are followed Round Robin study, which concludes in Table 2.5 (Faksness *et al.*, 2002).

Table 2.5 Recommended criteria for correlation studies of diagnostic ratios

Classification	Definition
Positive match	All DRs within the CL 95 %
Possible match	All DRs within the CL 98 % CL
No match	Any key DRs outside of CL 98 %

Note DRs = Diagnostic ratios, CL = Confidence limit

According to the Round Robin study, the comparison of the result of the spill sample and suspected source(s) are illustrated in x-y plots, linear regressions, and conclusions based on the “fit” between spill and source samples for the selected suite of diagnostic ratios can be made.

The straight line ($x=y$) can be concluded as the perfect match only when all DRs of the spill samples are the same as the suspected source oil. If the error bars of all DRs overlap the straight line, the spill sample is concluded to be a positive match to the suspected source oil, as shown in Figure 2.16 and Figure 2.18. Unless

one of the DRs does not overlap the line $x=y$, it could be concluded non-match to the source oil, as shown in Figures 2.17 and Figure 2.19.

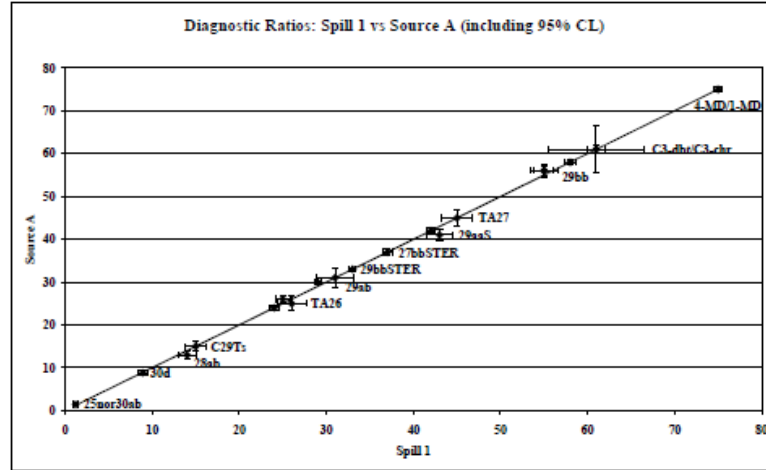


Figure 2.16 Correlation between spill 1 and source A, using a 95% confidence limit (positive match).

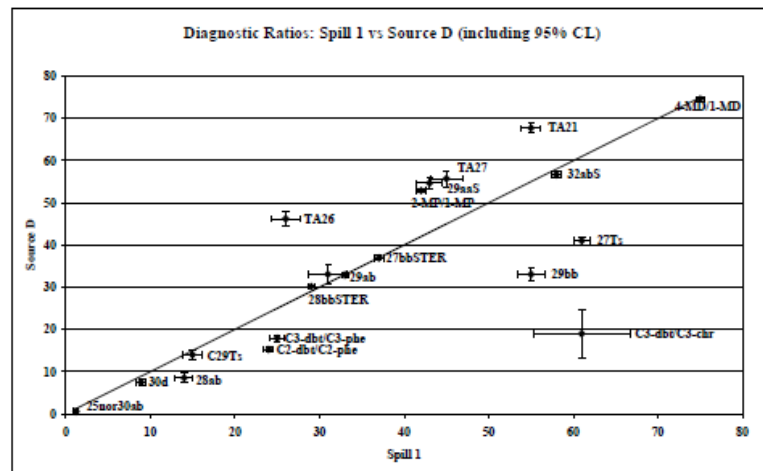


Figure 2.17 Correlation between spill 1 and source D, using a 95% confidence limit (non-match).

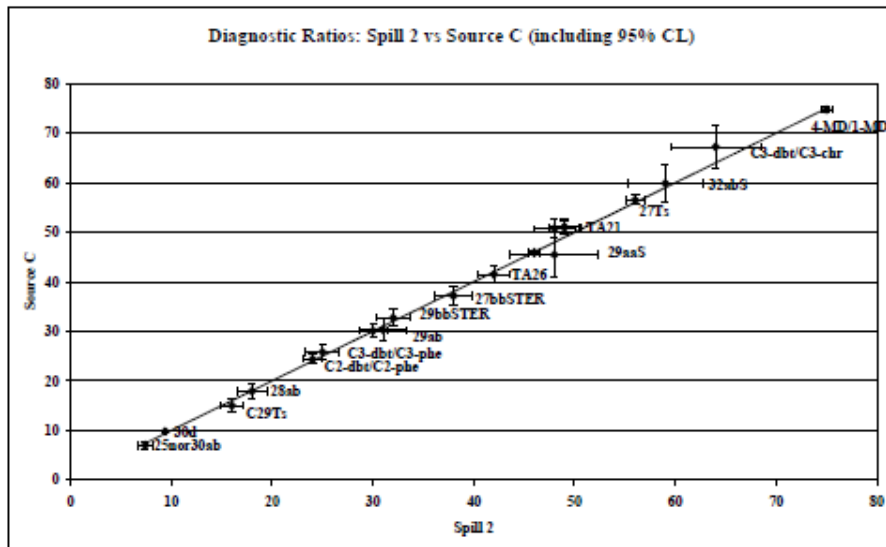


Figure 2.18 Correlation between spill 2 and source C, using a 95% confidence limit (positive match).

The use of different confidence intervals such as 95 %, 98 %, and 99 % confidence level is shown in Figures 2.19, Figure 2.20, and Figure 2.21, respectively. It shows that some of the ratios are still outside the straight line, although it was increased to 98 % or 99 %, which gave the same conclusion: non-match (Faksness *et al.*, 2002).

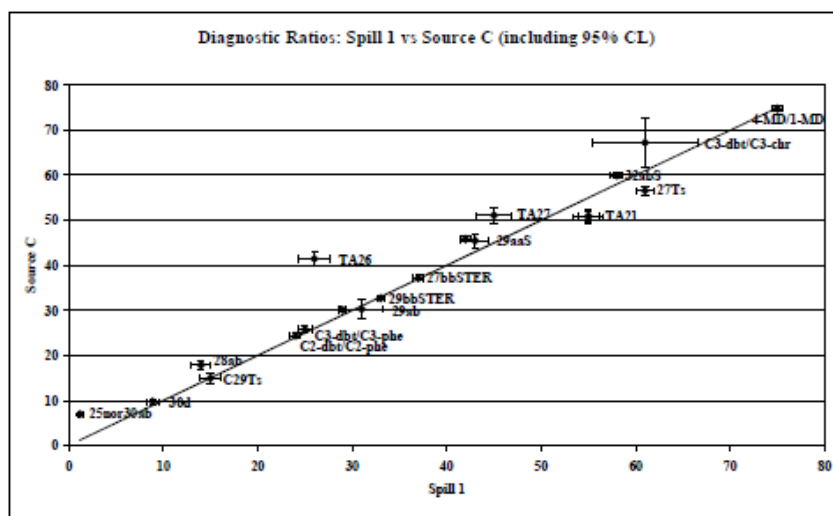


Figure 2.19 Correlation between spill 1 and source C, using a 95% confidence limit (non-match).

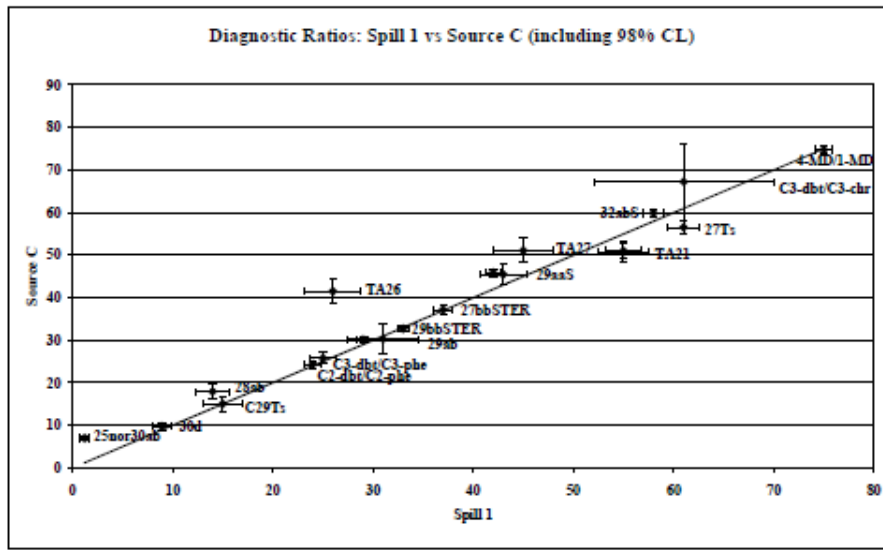


Figure 2.20 Correlation between spill 1 and source C, using a 98% confidence limit (non-match).

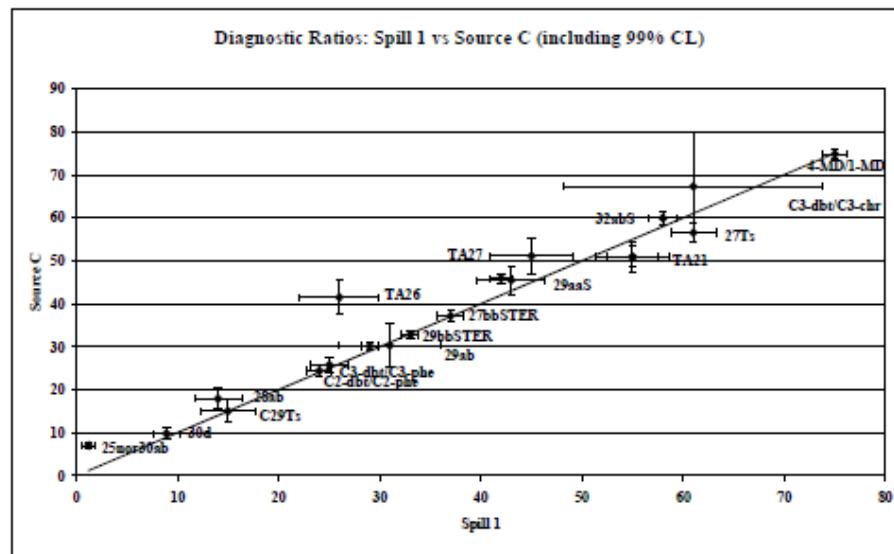
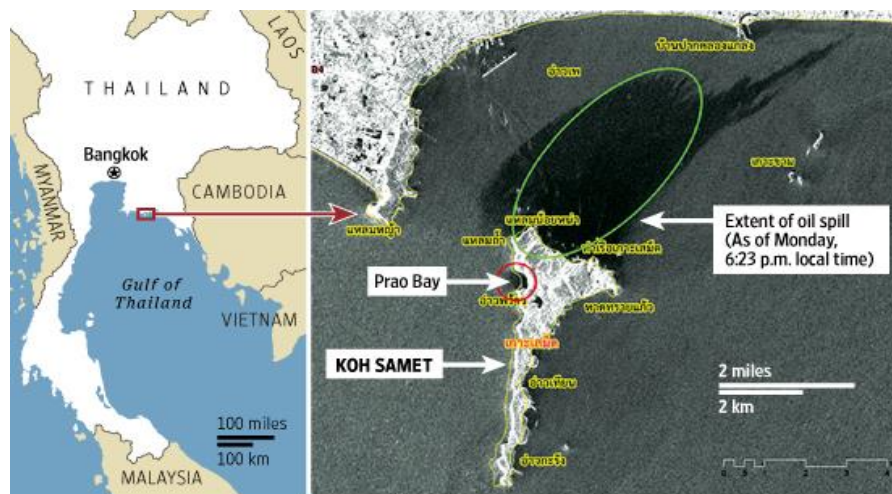


Figure 2.21 Correlation between spill 1 and source C, using a 99% confidence limit (non-match).

2.6 Example of Oil Spill in Thailand

There was an oil spill accident in the Gulf of Thailand on 27 July 2013. Rayong Province is confronted with a large oil spill problem. The accident was caused by the pipeline leakage owned by PTTGC plc. It was broke while transferring oil from tanker to refinery. At least 50,000 tonnes of crude oil were released into the sea and moved from the north of Koh Samet island, covering the Ao Phrao and other beaches. This accident caused two beaches to be closed, the tourists were evacuated, and marine animals, including coral reefs, to be contaminated. Figure 2.22 illustrates the approximate oil spill location in the Gulf of Thailand and the satellite picture of the extended area of the oil spill (Maierbrugger, 2013). Another oil spill case happened in Rayong Province recently. On 25 January 2022, an underwater crude oil pipeline owned by Star Petroleum Refining Public Company Limited (SPRC) was leaked. Figure 2.23 shows a satellite picture taken on 27 January 2022 by Geo-Informatics and Space Technology Development Agency.



Satellite Image: Geo-Informatics and Space Technology Development Agency, Thailand

Figure 2.22 Approximate location of the oil spill in Rayong, 2013 (Maierbrugger, 2013).

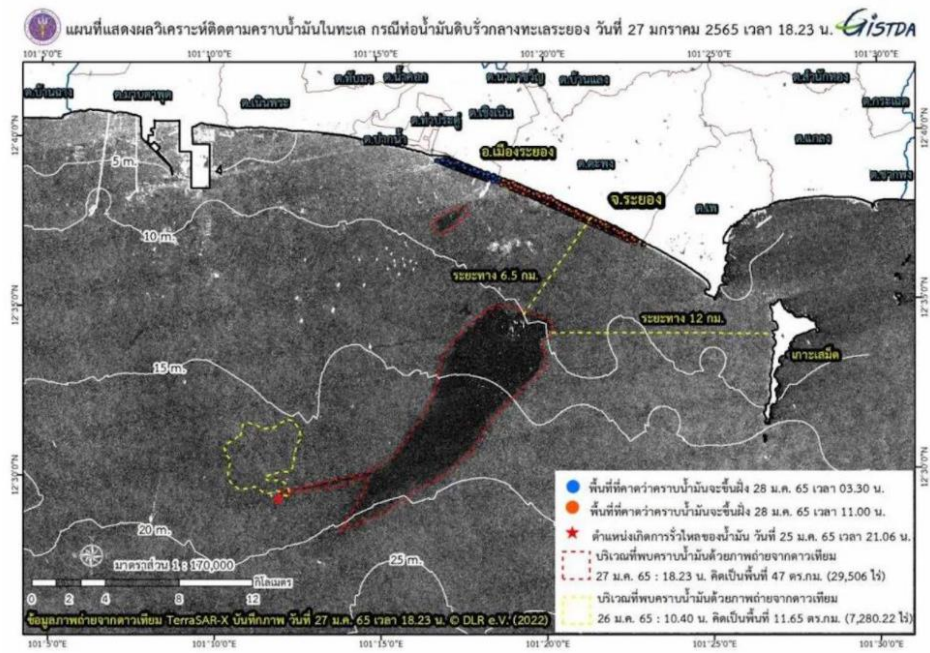


Figure 2.23 Approximate location of the oil spill in Rayong, 2022.

CHAPTER 3 EXPERIMENTAL

3.1 Material and Equipment

3.1.1 Oil Samples

The oil samples used in this research are given in Table 3.1. All crude oil samples (CO1-CO5) are commonly used as a feedstock for refining and they are classified as sour crude oil. The two fuel oil samples (FO1-FO2) are used as marine fuel.

Table 3.1 Information of crude oil and fuel oil samples

No.	Code	Sample Name	Description
1	CO1	Kissanje Crude	Crude oil, Angola, Africa
2	CO2	WTI Midland crude	Crude oil, USA
3	CO3	Kikeh Crude	Crude oil, Malaysia
4	CO4	Saharan Crude	Crude oil, Algeria, Africa
5	CO5	Doba Blend Crude	Crude oil, Cameroon, Africa
6	FO1	Fuel Oil 1	Fuel Oil
7	FO2	Fuel Oil 2	Fuel Oil

3.1.2 Gases

- The ultra-high purity (UHP) nitrogen for GC-TOFM
- The high purity (HP) helium is 99.995% for GC-TOFMS

3.1.3 Chemicals

- n-Hexane AR from RCI Labscan
- Dichloromethane from Burdick Jackson
- Silica gel from Merck, Germany
- Sodium carbonate sulfate from Asia Pacific Specially Chemicals Limited, Australia

- Sodium hydroxide AR grade from RCI Labscan
- Hydrochloric acid 37 wt% was purchased from EMSURE
- N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Merck
- 17 β (H), 21 β (H)-hopane solution from Fluka

3.1.4 Equipment

- Agilent© 7890 comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS)
- Nylon syringe filter 0.45 μ m and 0.2 μ m

3.2 Methodology

3.2.1 Weathering Simulation

The weathering process was simulated in a transparent container. The size of the container is 10 cm long, 10 cm wide, and 30 cm high, and 2 L of synthetic seawater is filled into each clean container. The synthetic seawater used in this experiment was made from reef salt with a salinity of 35 ppt. Then about 10 mL of oils were poured into the seawater surface. The containers were placed outside the building during the weathering process to expose to sunlight for 60 days. All the samples were stirred every 7 days. Weathered oils were sampled and analyzed on day 0, day 7, day 30, day 45, and day 60.

3.2.2 Extraction of Oil Sample

Biomarker compounds and naphthenic acid compounds were analyzed in this research. Therefore, there are 2 extraction methods prior to analysis.

3.2.2.1 *Biomarker Extraction*

Prior to GC analysis, 0.20-0.25 g of oil samples were placed into a 20 mL vial. High purity n-hexane/dichloromethane (1;1, v/v) 10 mL were added to the vial to extract the oil, and 1 g of anhydrous sodium sulfate was added to remove the moisture from the samples. The mixture was vortexed for 5 min and settled at room temperature for 4 h. The supernatant in the vial was filtered through a

0.45 μm nylon syringe filter to another vial. Then 1 g of silica gel was added to the vial. The mixture was vortexed for 2 min and settled for 2 min. Finally, the samples were filtered through a 0.2 μm nylon syringe filter and were transferred to a vial. For GC analysis, 200 μL of the extract was placed in a 2 mL vial and added with 1 μL 17 β (H), 21 β (H)-hopane as GC standard (Ho *et al.*, 2015; Zhang *et al.*, 2015).

3.2.2.2 Oxygenated Compounds Extraction

In order to extract the oxygenated compounds for analysis, 0.20-0.25 g of oil samples were placed into a 20 mL vial and then rinsed with 0.1 M NaOH 10 mL to dissolve the polar compounds and vortexed for 10 min. Samples were settled at room temperature for 4 h. Furthermore, the oil phase and water phase were separated. The supernatants (water phase) were acidified to pH < 2 using HCl and extracted three times with dichloromethane (v/v= 1:1). The extracts were pooled and dried with anhydrous Na_2SO_4 and filtered through 0.2 μm nylon syringe filter. The extracts were allowed to evaporate for 10 minutes to remove the solvent. The oxygenated compounds were derivatized with N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), and heated at 60 $^\circ\text{C}$ for 20 min. The sample was then added with 1 mL of hexane before analysis with GC-TOFMS (Grewer *et al.*, 2010; Zan *et al.*, 2019).

3.2.3 Analysis of Oil Sample Using GC-TOFMS

The extracted oil samples were analyzed by a LECO Pegasus 4D GC-TOFMS using the following GC condition:

Inlet:	Spitless
Detector:	Mass spectrometry
Column:	First dimension (1D): 60 m long x 0.25 mm internal diameter, 0.25 μm film thickness, capillary RTX-PAH column. Second dimension (2D): 1 m long x 0.25 mm internal diameter, 0.25 μm film thickness, capillary RTX-1HT column.
Gas:	Helium 1 mL/min
Temperature:	Injection port 300 $^\circ\text{C}$
Transfer line	300 $^\circ\text{C}$
MS source	250 $^\circ\text{C}$

Injection ratio: 5:1 split

The 1D oven conditions were isothermally at 40 °C for 1 min and increased the temperature to 340 °C with ramped at 2.5 °C/min, hold time 5 min.

3.3 Data Collection and Analysis

3.3.1 ChromaTOF Software

Data is acquired and processed using LECO ChromaTOF-GC software. PAHs, hopane groups, and oxygenated compounds analysis were set up to resample with a mass range of 45 to 550 atomic mass units with an acquisition rate of 200 spectra/sec.

The mass to charge for oxygenated compounds (m/z) is 73 which is the secondary fragments formed from iodized dimethylsiloxy group from derivatives agent BSTFA reacted with parent compounds. However, hopane group has a specific mass to charge (m/z) 191.

Diagnostic ratios were calculated using this equation,

Ratio = $100 \cdot A / (A+B)$, where the value for A and B was calculated by (area of the analyte)/(area of internal standard).

The chemical structure of the components is also provided in this software. From this information, acyclic and cyclic oxygenated compounds can be distinguished. The acyclic/cyclic ratios were calculated using the following equation:

$$A/C \text{ Ratio} = \frac{\sum NAs_{Z=0}}{\sum NAs_{Z=-2,-4,-6,-8}}$$

where Z is the hydrogen deficiency. Z=0 is acyclic aliphatic acid, Z=-2 is monocycle carboxylic acids, and dicyclic-, tricyclic, and tetracyclic carboxylic acids for Z=-4,-6,-8, respectively.

3.3.2 Microsoft Excel

Microsoft Excel was used to calculate diagnostic ratios using the statistical theory that concerns the confidence interval relative to the student's t

constant value, which depends on the number of observations.

Distribution plots of naphthenic acid classes were made with this software. The relative abundance of each class was calculated by determining the total area of each class and divided by the total area of all oxygen classes.



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CHAPTER 4

RESULTS AND DISCUSSION

This Chapter shows the result of the fresh and weathered samples, which are 5 crude oil samples, including Kissanje crude, WTI Midland crude, Kikeh crude, Saharan crude, Doba Blend crude (CO1-CO5), and 2 fuel oil samples (FO1 and FO2). The physical appearance of the samples before and after weathering was observed. Moreover, the distribution of biomarkers and the naphthenic acid compound is discussed in this Chapter.

4.1 Weathering of Oil Samples

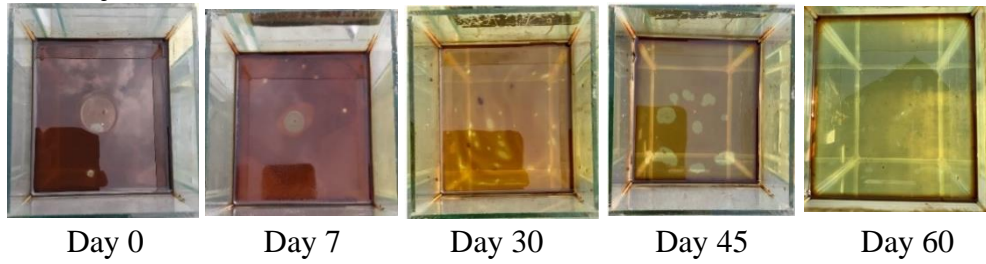
The weathering of crude oils and fuel oils in synthetic seawater was observed daily.

4.1.1 The Appearance of Oil in Seawater

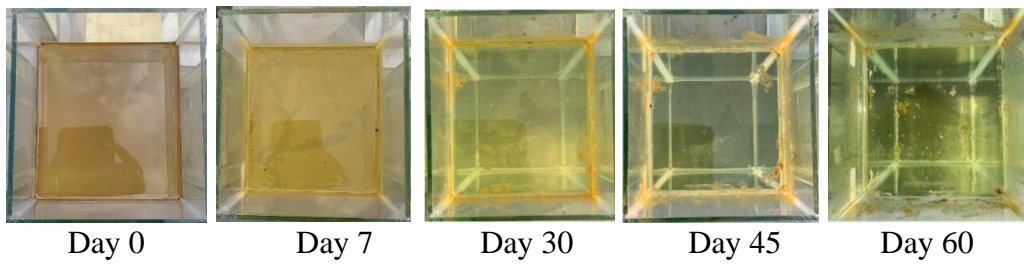
4.1.1.1 *Crude Oil*

Figure 4.1 (a)-(e) demonstrates crude oil's appearance during the weathering simulation process. All crude oils spread immediately over the seawater on Day 0. Black crude oils are very heavy, while the other color, greenish-yellow and reddish, has other non-hydrocarbon components (e.g., sulfur) that change their color. After 7 days, all crude oil spread over the seawater. Fading of oil over the surface was observed due to the evaporation process. On day 30, day 45, and day 60, the amount of crude oil was significantly lost, and some oil stuck on the container wall. Moreover, all crude oil appeared more viscous due to the evaporation of the light component.

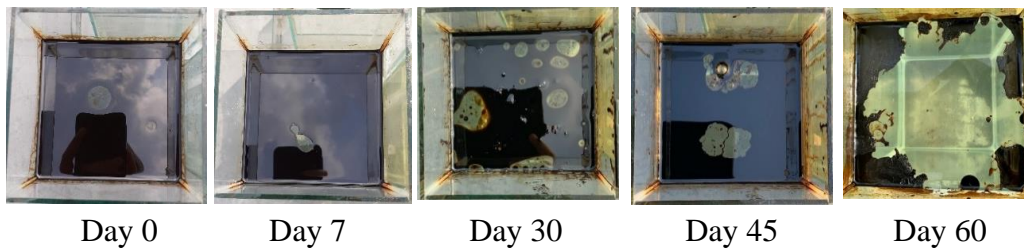
(a) Kissanje Crude oil



(b) WTI Midland Crude Oil



(c) Kikeh Crude Oil



(d) Saharan Crude Oil

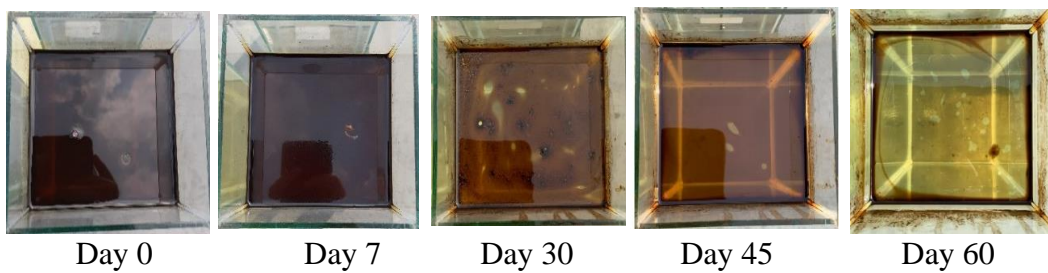


Figure 4.1 The physical appearance of crude oil samples in seawater on day 0, 7, 30, 45 and 60.

(e) Doba Blend Crude Oil

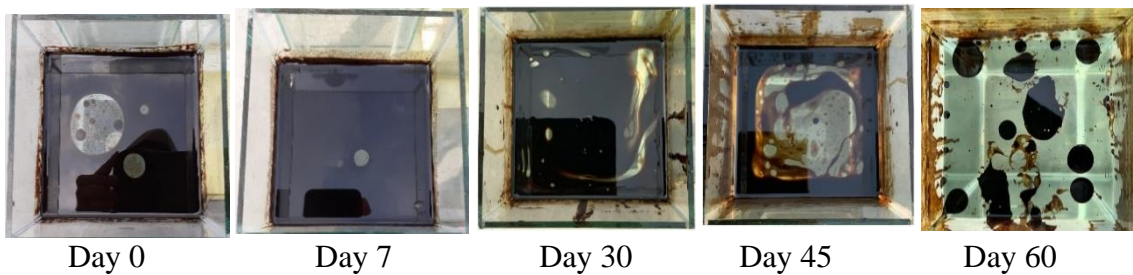
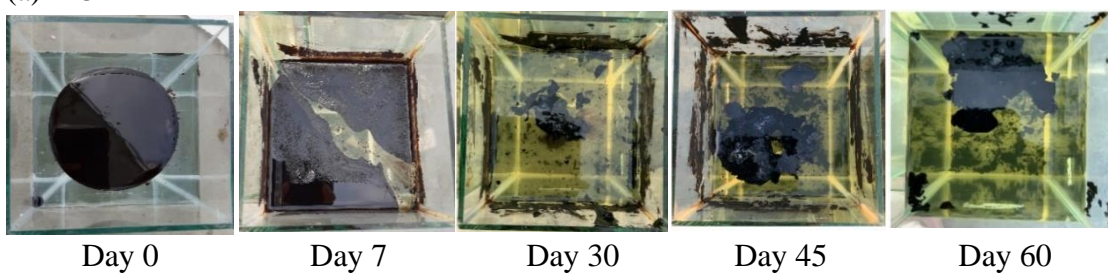


Figure 4.1 (cont.) The physical appearance of crude oil samples in seawater on day 0, 7, 30, 45 and 60.

4.1.1.2 Fuel Oil

Figure 4.2. (a) and (b) shows the physical appearance of fuel oil during the weathering simulation. On day 0, all fuel oil samples had the same physical appearance that was not spread instantly after the oil spill and were formed a thick layer on the seawater surface. After 7 days of the oil spill, FO1 and FO2 spread over the seawater surface. During day 30, day 45, and day 60, all fuel oil samples had a similar physical appearance, which is observed as more viscous than weathered crude oil. Moreover, there were fuel oil samples that stuck on the container surface. FO1 and FO2 were looked like tarballs at day 60.

(a) FO1



(b) FO2

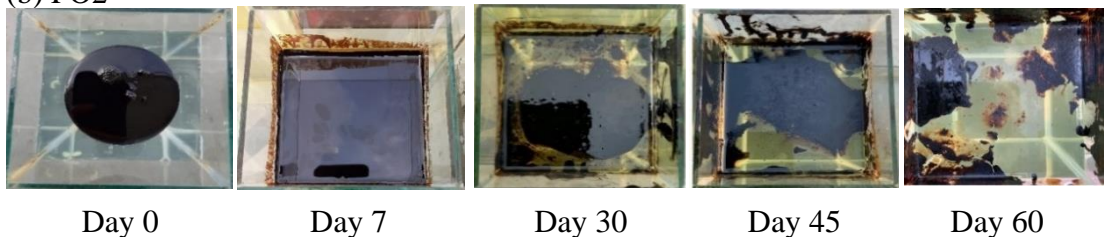


Figure 4.2 The physical appearance of fuel oil samples in seawater on day 0, 7, 30, 45 and 60.

4.2 Analysis of Oxygenated Compounds

4.2.1 Oxygenated Compounds Distribution from GC-TOFMS

The analysis of oxygenated compounds in crude oil and fuel oil was investigated using the GC-TOFMS instrument. Total ion chromatograms (TICs) for fresh and weathered crude oil and fuel oil samples are presented in Figures 4.3 to 4.6. The chromatograms pattern of all fresh crude oils and fuel oil were varied. The oxygen-nitrogen-containing compound, $C_{22}H_{43}NO$, was found in fresh oil samples and day 7 samples. Visualization analysis of chromatograms indicated that the low molecular weight compounds that appeared at the earlier retention time, 500 s to 3000 s, were affected by weathering after 7 days. Light components in crude oil and fuel oil were dissolved and lost during evaporation. Figures 4.5 and 4.6 show the TICs of crude oil and fuel oil after 60 days of weathering. The chromatograms show that the noise detection is greater than day 0 and day 7 samples. The intensity of the peak also decreased. This indicates that oxygenated compounds concentration in all samples decreased significantly after 60 days of weathering.

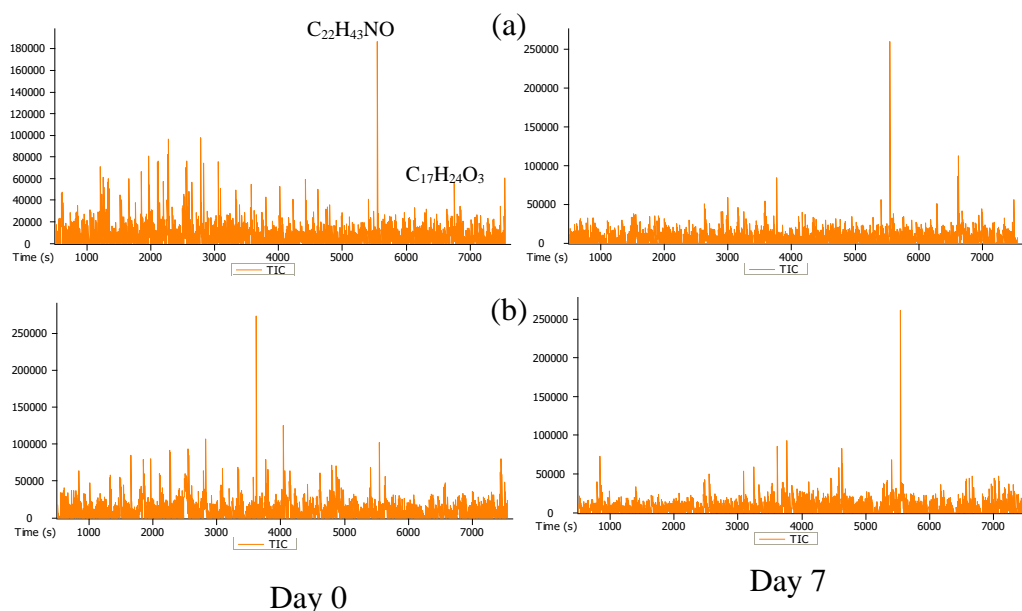


Figure 4.3 Total ion chromatograms on day 0 and day 7 of (a) Kissanje crude oil; (b) WTI Midland crude oil; (c) Kikeh crude oil; (d) Saharan crude oil; and (e) Doba Blend crude oil.

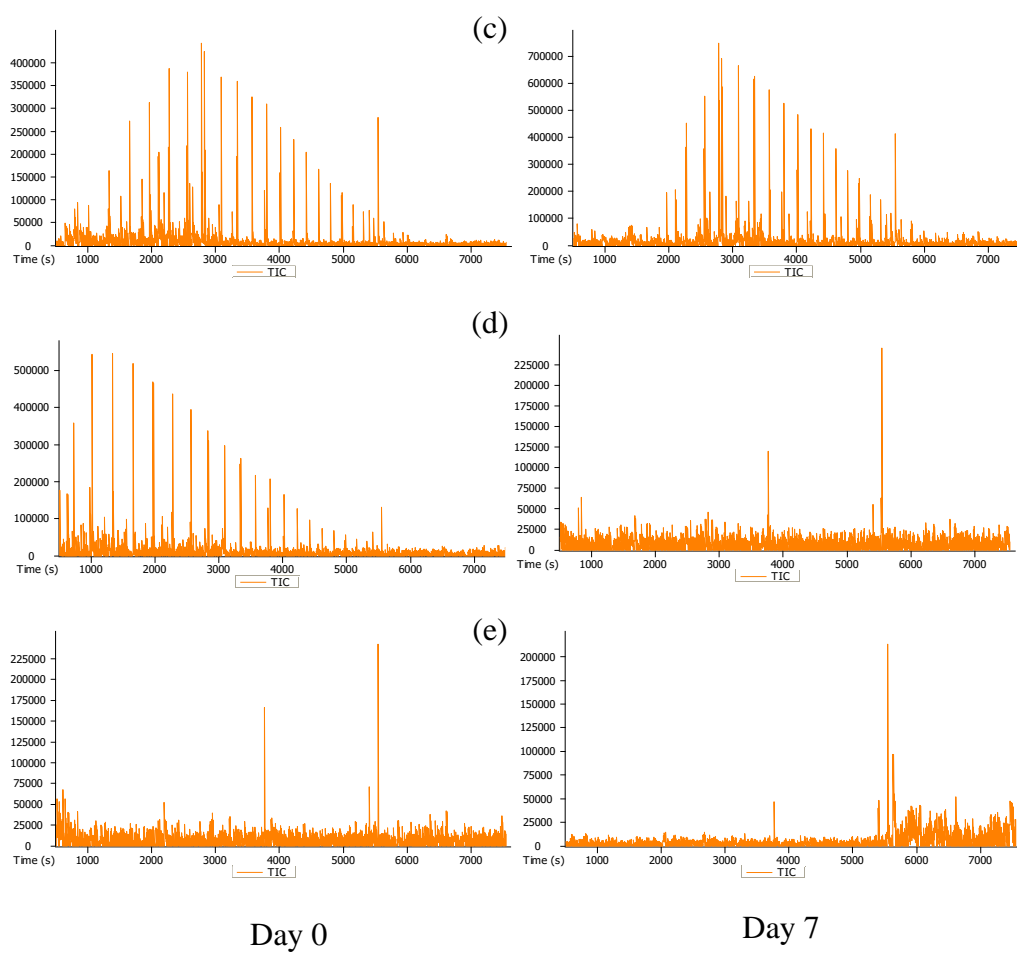


Figure 4.3 (cont.) Total ion chromatograms on day 0 and day 7 of (a) Kissanje crude oil; (b) WTI Midland crude oil; (c) Kikeh crude oil; (d) Saharan crude oil; and (e) Doba Blend crude oil.

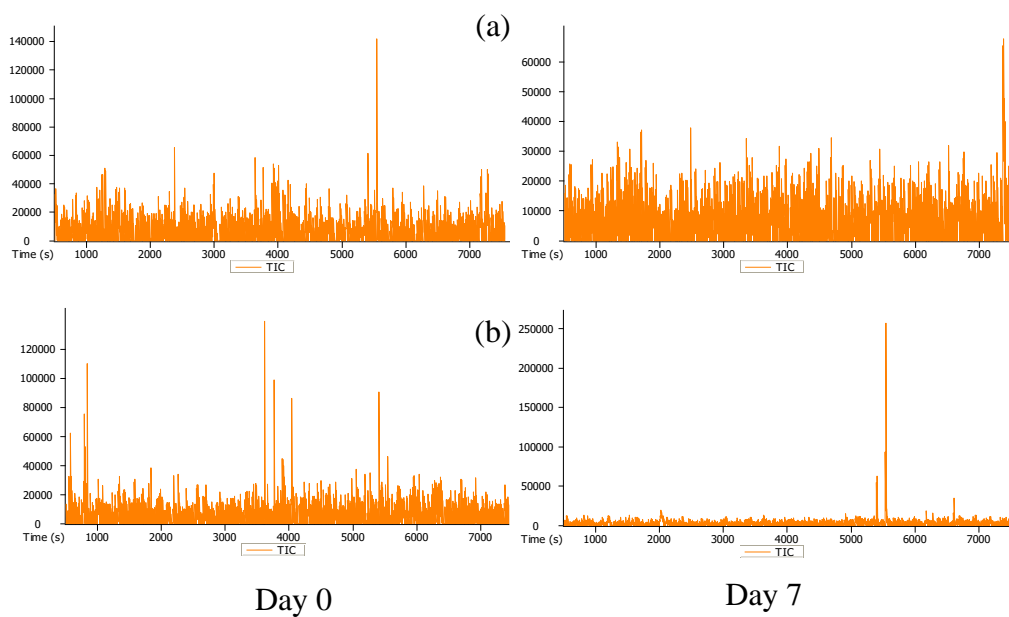


Figure 4.4 Total ion chromatograms on day 0 and day 7 of (a) Fuel oil 1 and (b) Fuel oil 2.

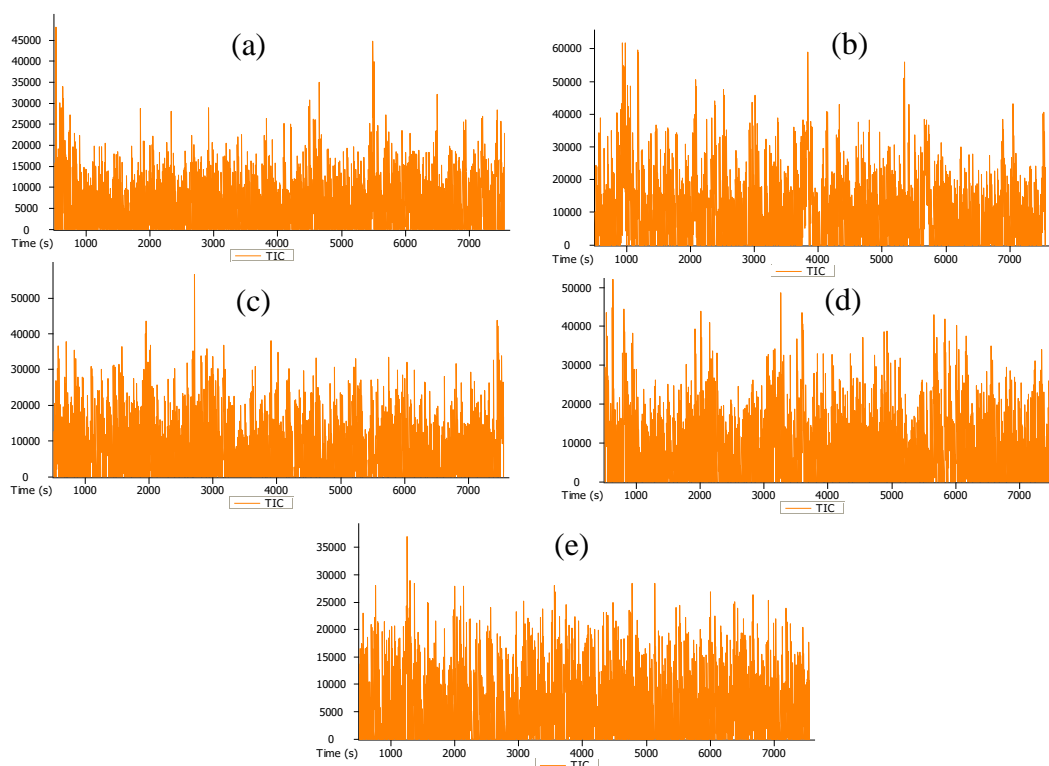


Figure 4.5 Total ion chromatograms on day 60 of (a) Kissanje crude oil; (b) WTI Midland crude oil; (c) Kikeh crude oil; (d) Saharan crude oil; and (e) Doba Blend crude oil.

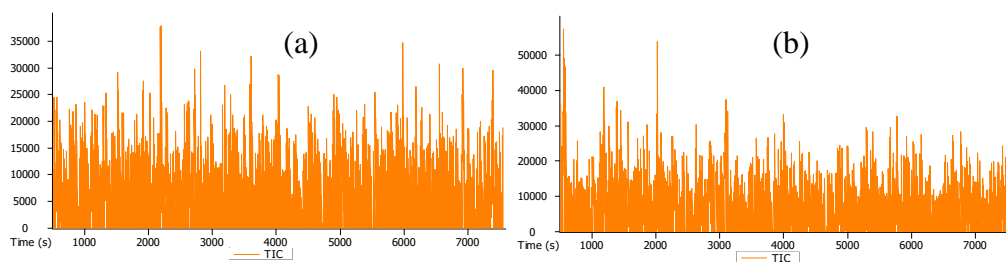


Figure 4.6 Total ion chromatograms on day 60 of (a) Fuel oil 1 and (b) Fuel oil 2.

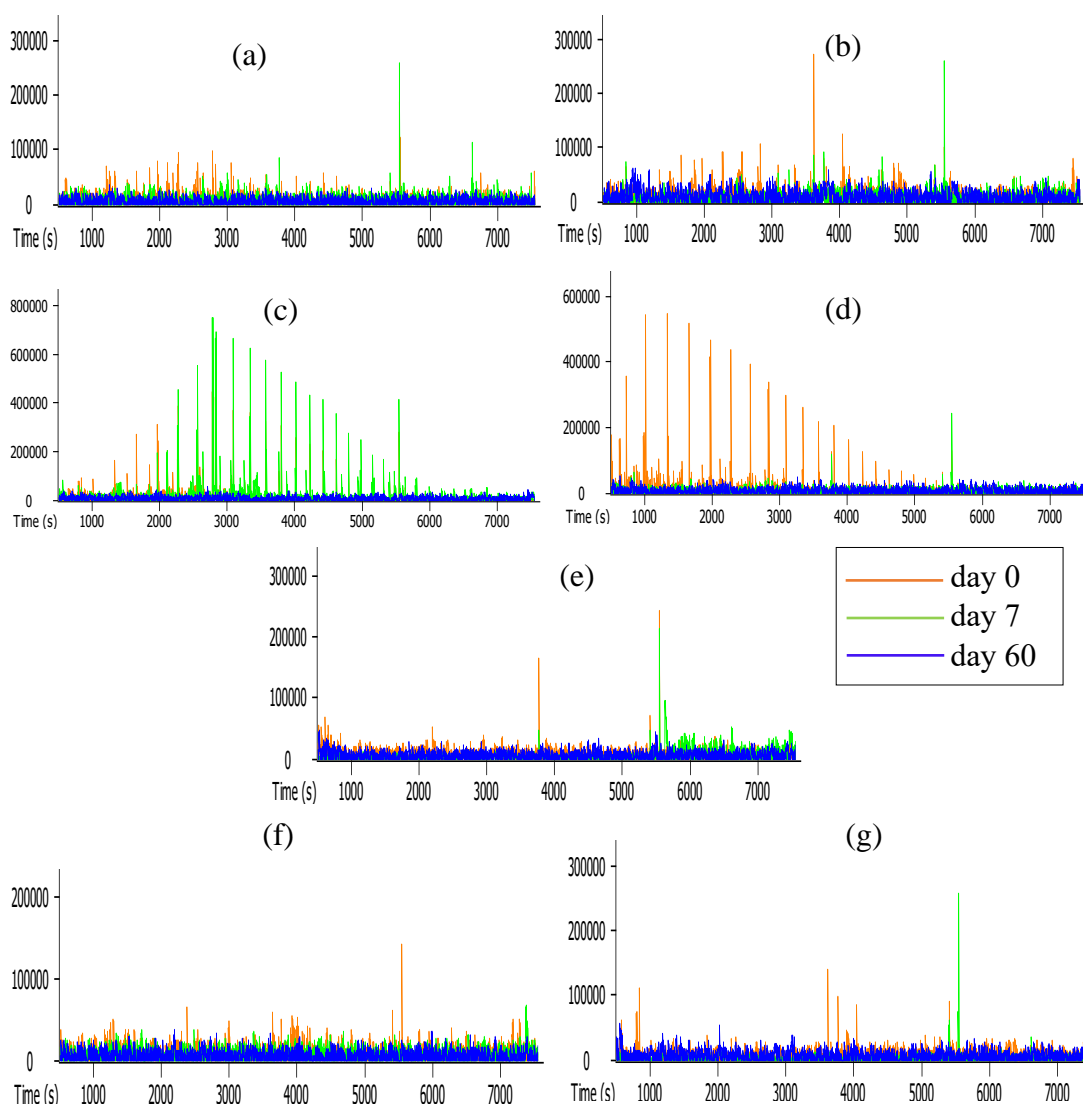


Figure 4.7 Overlay TIC chromatograms of (a) Kissanje crude oil; (b) WTI Midland crude oil; (c) Kikeh crude oil; (d) Saharan crude oil; (e) Doba Blend crude oil; (f) Fuel oil 1; and (g) Fuel oil 2.

4.2.2 Oxygenated Compounds Identification by GC-TOFMS

The extracted samples were derivatized using BSTFA as the silylation agent. The addition of the silylation agent in analysis resulted in the GC signals eluting later than the non-derivatized sample (Shepherd *et al.*, 2010). The mass to charge (m/z) 73 is secondary fragments formed from the molecular ion containing an iodized dimethylsiloxy group attached to the hydrocarbon portion of the parent compound (Poole, 2013). The peak of the oxygenated compound can be distinguished from m/z 73. The major component of petroleum naphthenic acids mixture is acyclic (Rowland *et al.*, 2011). The examples of acyclic acid compounds found in samples are $C_{17}H_{34}O_2$, $C_{18}H_{36}O_2$, $C_{19}H_{36}O_2$, and $C_{26}H_{50}O_4$. Cyclic compounds were also identified, the examples of cyclic compounds are $C_{16}H_{22}O_4$, $C_{20}H_{30}O_4$, $C_{22}H_{34}O_4$, and $C_{27}H_{42}O_4$.

Table 4.1 shows that the ratio of acyclic to cyclic naphthenic acid in fresh crude oil is >1 , indicating that the acyclic naphthenic acid compound is more abundant than cyclic naphthenic acid. However, the naphthenic acid compounds are not resistant to the weathering process due to biodegradation, photo-oxidation, evaporation, and dissolution. Zan, S., 2019., demonstrate that the concentration of NAs in sediment sample in the environment were 10-30 times higher than the concentration of total PAHs after 5 years of the Dalian Bay oil spill. Moreover, the acyclic to cyclic (A/C) naphthenic acid ratio decreased after 2 and 3 years. However, it showed a similar ratio to the origin crude oil after 4 years of the oil spill.

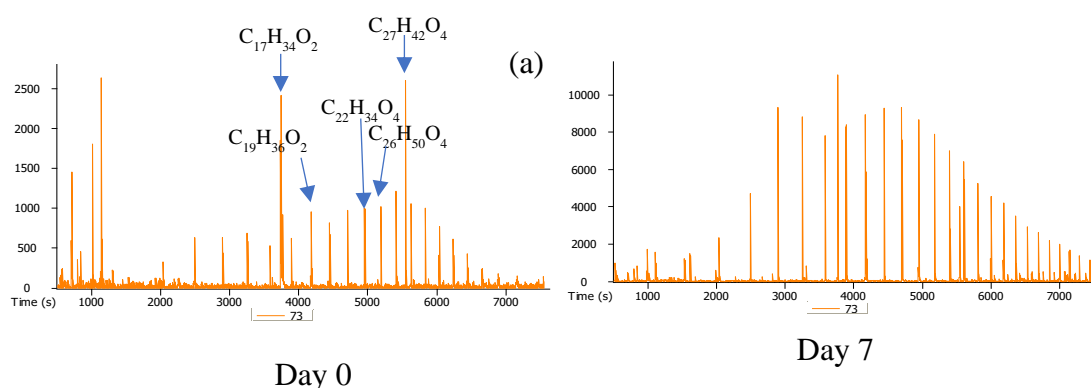


Figure 4.8 Selected ion chromatogram (m/z 73) on day 0 and day 7 of (a) Kissanje crude oil; (b) WTI Midland crude oil; (c) Kikeh crude oil; (d) Saharan crude oil; and (e) Doba Blend crude oil.

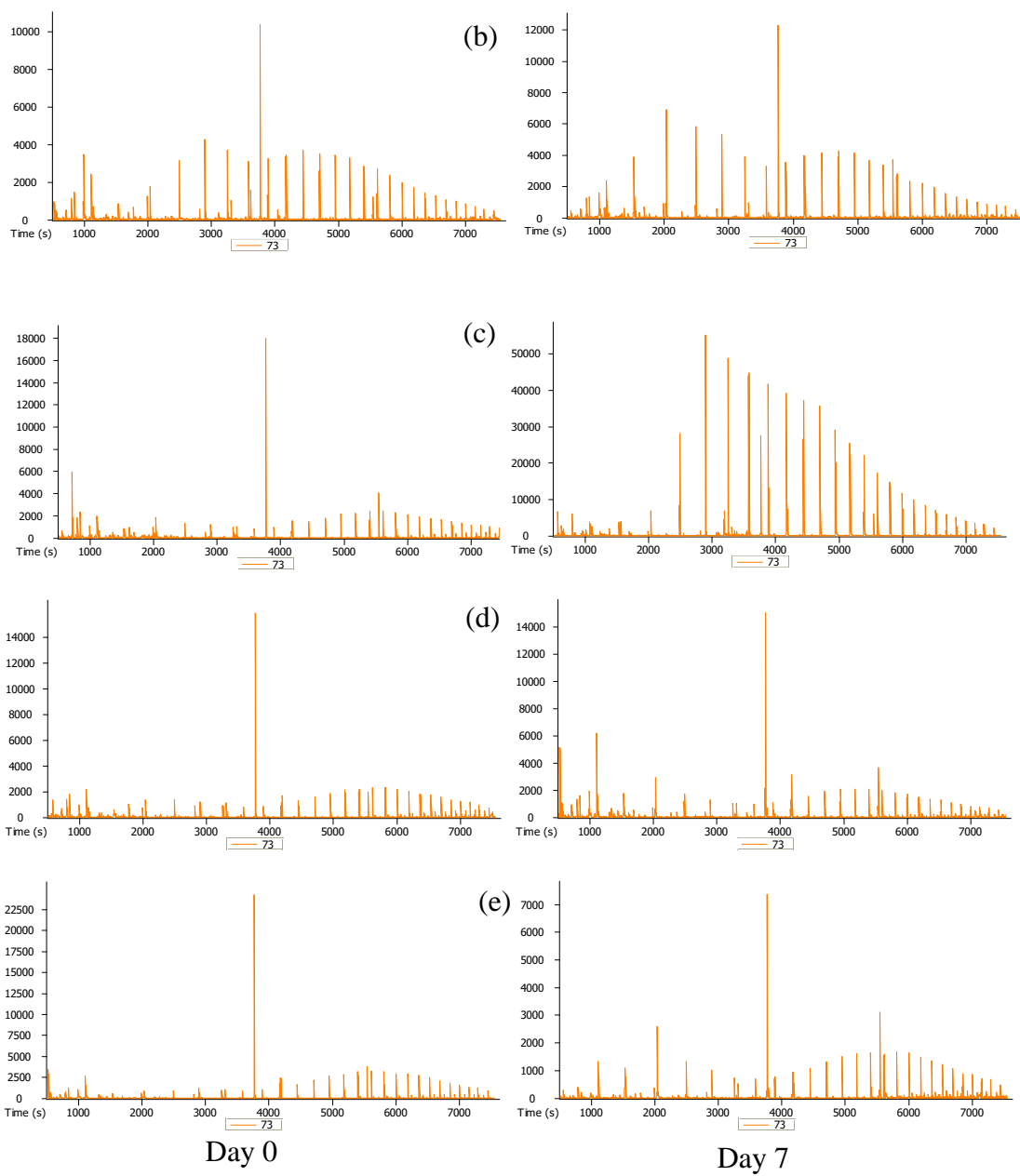


Figure 4.8 (cont.) Selected ion chromatogram (m/z 73) on day 0 and day 7 of (a) Kissanje crude oil; (b) WTI Midland crude oil; (c) Kikeh crude oil; (d) Saharan crude oil; and (e) Doba Blend crude oil.

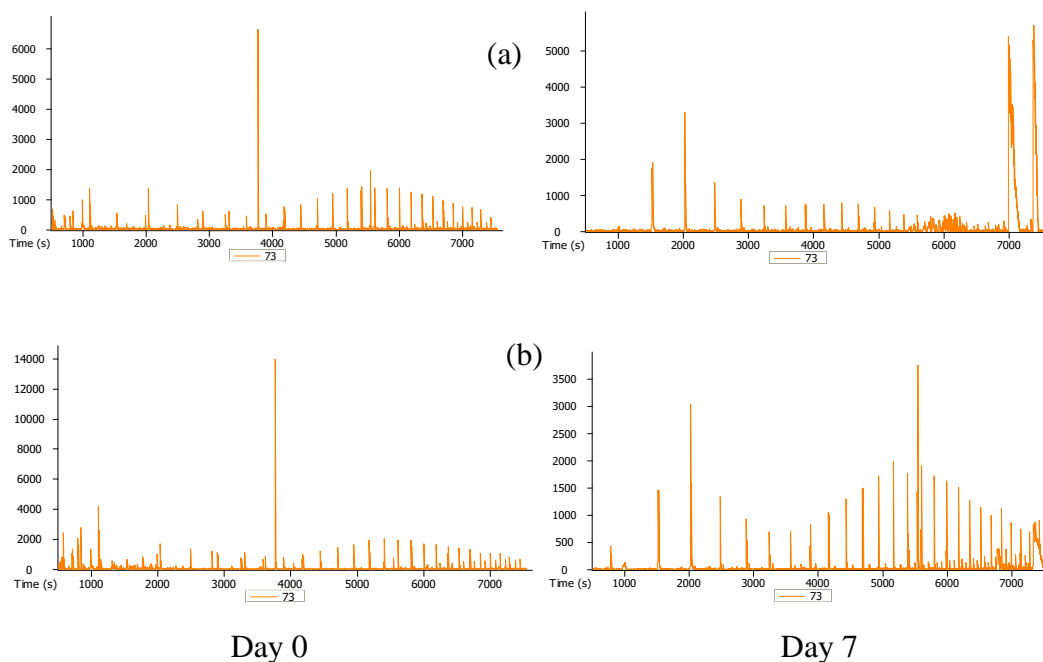


Figure 4.9 Selected ion chromatogram (m/z 73) on day 0 and day 7 of (a) Fuel oil 1 and (b) Fuel oil 2.

The abundance and distribution of heteroatom classes varied between samples. Figures 4.10 and 4.11 show the comparison of class distribution of naphthenic acid from Kissanje crude oil and Doba Blend crude oil. Nitrogen-oxygen-containing compound are the most abundant class in fresh crude oil samples. However, it decreased significantly after the weathering process. The relative abundance of O₁, O₂, and NO classes decreased after 7 days while the O₃ and O₆ increased. However, the relative abundance of O₄ class slightly decreased for Kissanje crude oil while significantly increased in Doba blend crude oil. Microbial metabolism and sunlight can add oxygen to the oil component and form higher order oxygen classes compound (O₄-O₆) (Ray *et al.*, 2014; Whitby, 2010). The addition of oxygen in the component will increase carboxylic acid content, which may decrease the NAs hydrophobicity and thus become more bioavailable. At the same time, it can decrease the relative abundance of O₁, O₂, and O₃ classes (Yue *et al.*, 2016).

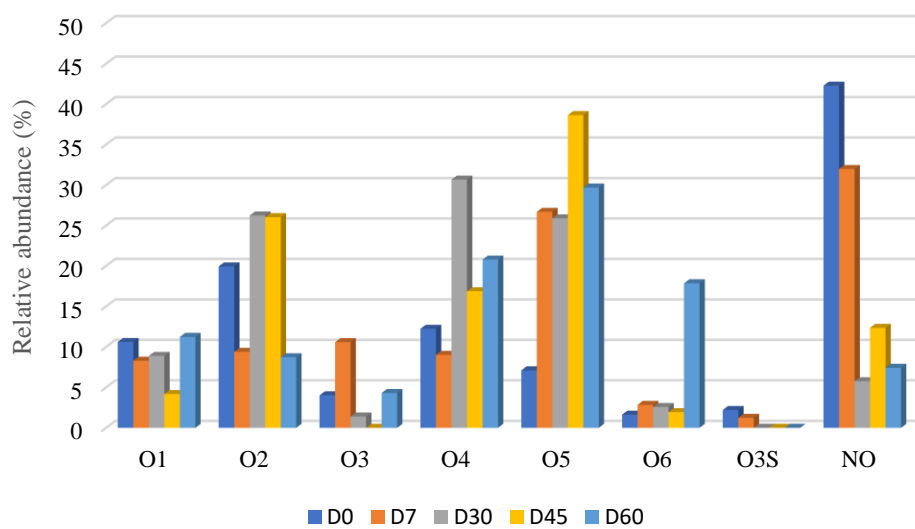


Figure 4.10 Distribution plots of naphthenic acid classes from Kissanje crude oil.

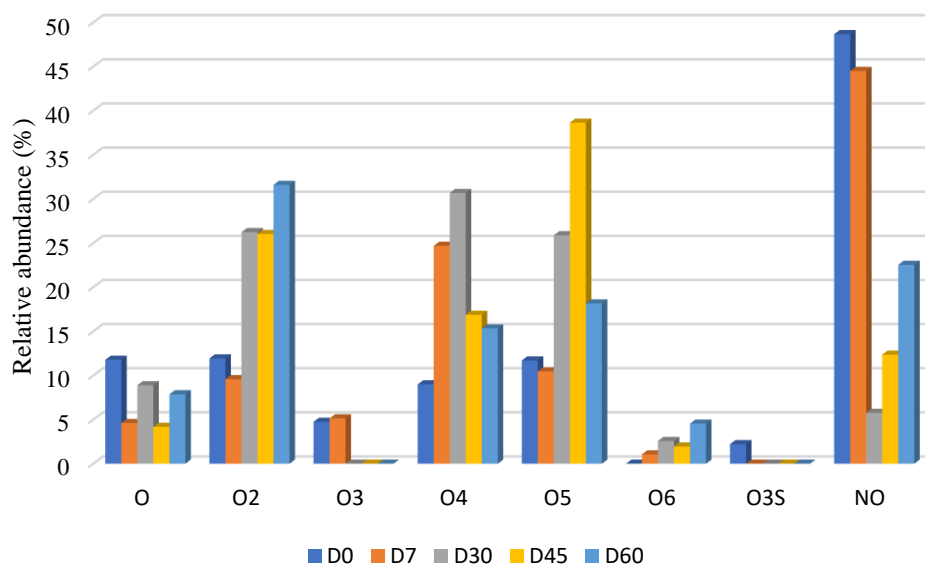


Figure 4.11 Distribution plots of naphthenic acid classes from Doba Blend crude oil.



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4.2.3 Differentiation of Crude Oil and Fuel Oil Using Acyclic/Cyclic Naphthenic Acid Ratio

The types of NAs included acyclic aliphatic acid and cyclic acid. Naphthenic acid species with one double bond equivalent (acyclic compound) dominate the distribution for non-degraded oil. Generally, acyclic acids can be degraded more rapidly than cyclic compounds in the environment. This results in a consistent increase in cyclic NAs species and a decrease in acyclic acids, suggesting that these compounds could be used to measure the degree of biodegradation (Kim *et al.*, 2005). The results of acyclic to the cyclic naphthenic acid compound ratio (A/C) of fresh and weathered oil samples are shown in Table 4.1. The results show that the ratio decreased in all samples after weathering. Fresh crude oil samples (day 0) have the ratio between 1.02 to 3.20. However, the A/C ratio of fresh fuel oil (day 0) are 0.58 and 0.98. In general, weathered crude oil samples have a ratio of 0.67 to 2.59, except for Kissanje crude oil on day 60, which have a ratio of 0.47. The ratio of A/C for weathered fuel oil is <0.65. The differentiation of crude oil and fuel oil with an A/C ratio can be considered since fuel oil exhibit a lower A/C ratio than crude oil. Thus, the calculation of biomarker diagnostic ratio is needed to confirm the oil spill origin.

Table 4.1 The Acyclic to Cyclic Acid Compound Ratio

Sample Name	A/C Ratio		
	Day 0	Day 7	Day 60
Kissanje CO	1.02	0.67	0.47
WTI Midland CO	3.20	2.59	1.29
Kikeh CO	1.17	0.98	0.72
Saharan CO	1.17	0.95	0.86
Doba Blend CO	2.96	2.53	1.72
FO1	0.58	0.55	0.18
FO2	0.98	0.64	0.34

4.3 Analysis of Biomarkers

4.3.1 General Total Ion Chromatograms of Oil Sample

Oil samples were analyzed by GC-TOFMS. The weathering processes affected the chemical composition of the oil. Figure 4.12 illustrates the overlay chromatograms of Kissanje crude oil at day 0 and day 30. The light components suffer from weathering process, which indicates the disappearance of peak at retention time 0 to 4000 s after 30 days.

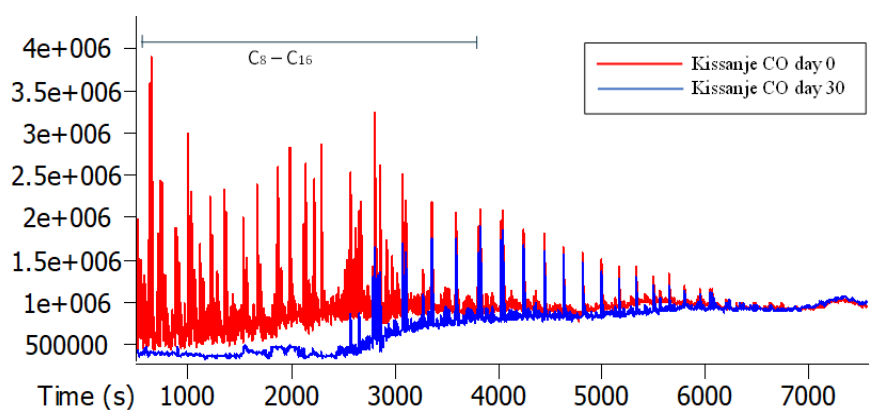


Figure 4.12 Total ion chromatogram (TIC) of Kissanje crude oil at day 0 and day 30.

4.3.2 The Hopane Group Distribution by GC-TOFMS

Figure 4.13. shows the hopane distribution of Kissanje crude oil before and after weathering for 30 days. The selected mass ion 191 illustrates that the distribution of the hopanes group before and after weathering has the same chromatogram pattern. This result confirms that the hopanes group resisted from weathering process. Hence, the hopanes group can be utilized as support identification of oil samples.

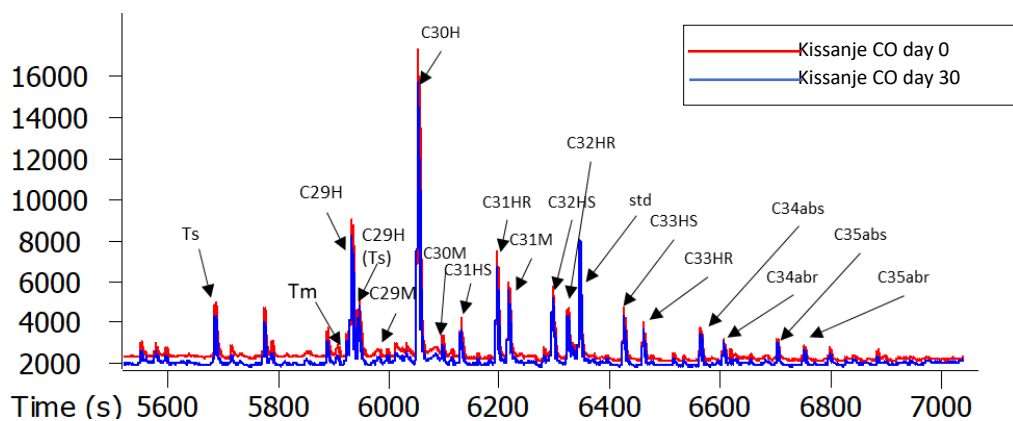


Figure 4.13 Selected ion chromatogram (m/z 191) of Kissanje crude oil at day 0 and day 30.

The hopane group distributions from the same crude oil region seem to have a similar pattern, as shown in Figure 4.14. The origin of Doba blend crude oil and Kissanje crude oil are from Africa. However, the different crude oil origin regions have different hopane distribution patterns, as shown in Figure 4.15. Kikeh crude oil is from Malaysia while Doba blend is from Africa. The diagnostic ratios were calculated to create the correlation plot to confirm the possible match of two different samples.

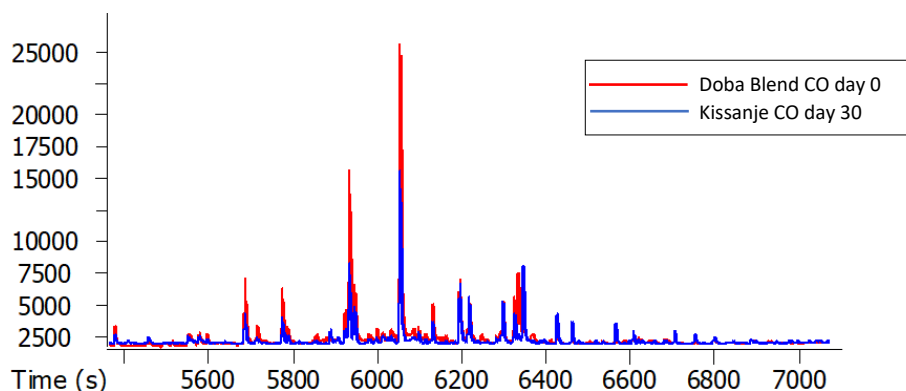


Figure 4.14 Selected ion chromatogram (m/z 191) of Doba blend crude oil at day 0 and Kissanje Crude oil at day 30.

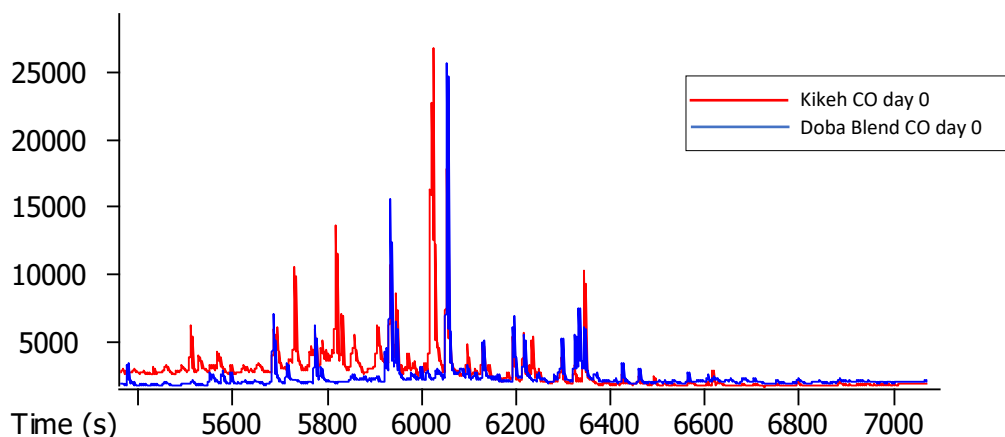


Figure 4.15 Selected ion chromatogram (m/z 191) of Kikeh crude oil at day 0 and Doba blend crude oil at day 0.

4.3.3 Identification of Oil Samples by Using Diagnostic Ratios

The diagnostic ratio of biomarker is a widely used technique for oil correlation, environmental forensic, and oil spill studies. The advantage of this technique is to reduce the concentration-effect while comparing the spilled oil and suspected source samples. The diagnostic ratio can be used to determine both quantitative (i.e., compound concentration) and semiquantitative data (i.e., peak areas or height) (Wang *et al.*, 2016). The accuracy of the instrument and the selection of biomarkers will indicate the reliability of diagnostic ratios. The peak area of the hopanes group is obtained from GC-TOFMS with selected mass ion 191 as the unique mass of the hopanes group. In this study, the diagnostic ratios were calculated from (area of biomarker 1) / (area of biomarker1 + area of biomarker 2). The diagnostic ratios result of biomarkers in crude oils is summarized in Table 4.2.

Table 4.2 The diagnostic ratio of the hopane group

Name of Biomarker	Kissanje CO		Doba blend CO	Kikeh CO
	D-0	D-30	D-0	D-0
Ts	13.4	16.7	19.0	43.3
Tm	4.5	4.2	13.6	39.9
C29H	32.4	32.0	8.3	67.3
C29H(Ts)	17.9	18.3	35.1	48.7
C29M	2.2	1.8	3.7	61.9
C30H	50.0	50.0	0.0	0.0
C30M	5.3	5.9	50.0	50.0
C31HS	11.6	11.4	10.9	18.1
C31HR	25.4	25.1	15.9	68.9
C31M	19.5	19.9	13.4	88.6
C32HS	21.7	22.0	2.1	76.0
C32HR	13.8	14.7	13.6	27.7
C33HS	14.3	15.7	12.5	35.5
C33HR	11.3	11.6	19.0	0.0
C34abs	10.0	10.8	0.0	0.0
C34abr	6.3	6.4	4.3	0.0
C35abs	6.7	8.0	3.3	0.0
C35abr	0.0	6.1	0.0	0.0

The diagnostic ratio from Table 4.2. is used to create the correlation plot. Figure 4.16. shows the correlation plot of Kissanje crude oil before and after weathering process for 30 days. The result shows that the 2 samples have a positive match, and it can indicate the resistance of the hopanes group to the weathering. The result as reported earlier by Mulabagal *et al.*, 2013 and Wang *et al.*, 2016 .

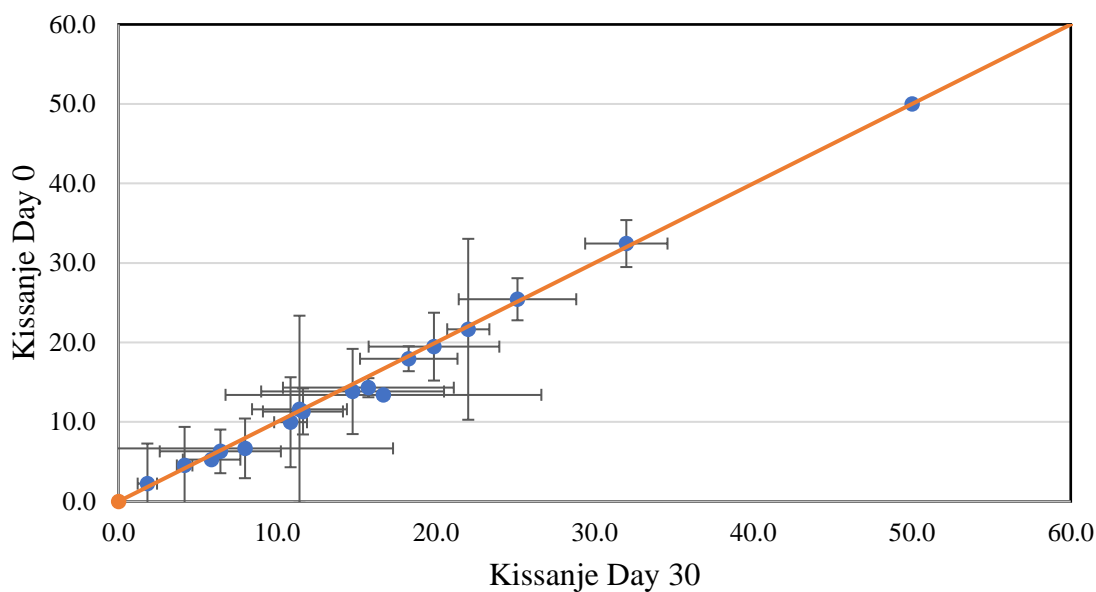


Figure 4.16 The correlation plot of Kissanje crude oil at day 0 and day 30, using a 95% confidence limit (Match).

Figure 4.17. shows the correlation plot of Kissanje crude oil day 30 and Doba blend crude oil, both from Africa. Some of the diagnostic biomarker ratios were overlapped with the linear line $x=y$. The conclusion from this plot is “non-match”. However, the correlation plot of Kikeh crude oil at day 0 and Doba blend crude oil at day 0, which come from different regions, shows that almost all the diagnostic biomarker ratios were not overlapped with the linear line. The result was “non-match”. Therefore, the hopanes group and the correlation method can be used to differentiate crude oils.

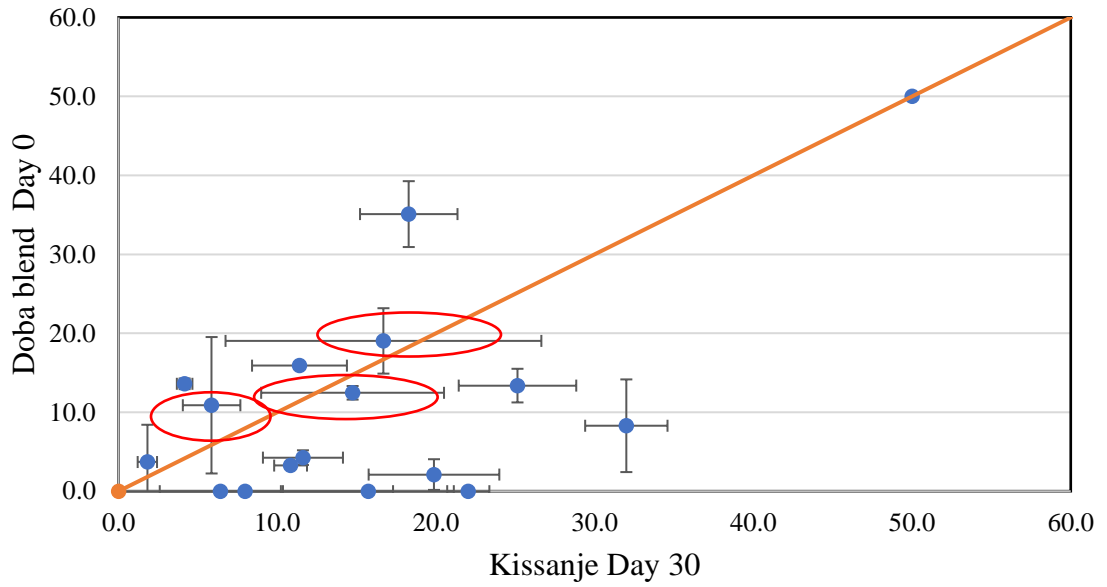


Figure 4.17 The correlation plot of Kissanje crude oil at day 30 and Doba blend crude oil at day 0 using a 95% confidence limit (non-match).

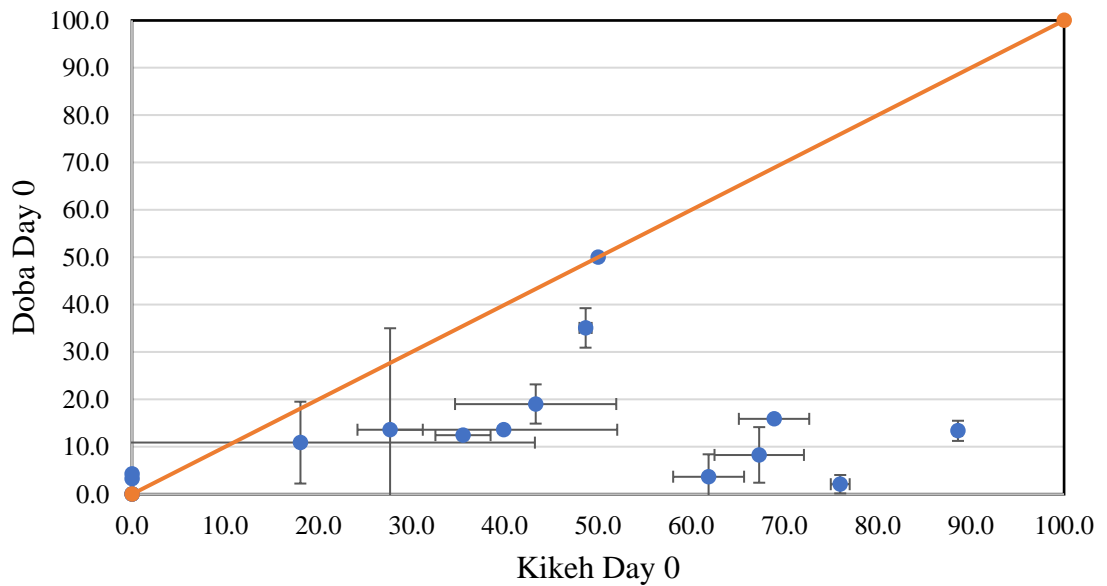


Figure 4.18 The correlation plot of Kikeh crude oil at day 0 and Doba blend crude oil at day 0 using a 95% confidence limit (non-match).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This work studied the distribution of the oxygenated compounds by using GC-TOFMS to support the identification of crude oil and fuel oil. Five crude oils and two fuel oils were investigated in this research. All samples were allowed to be weathered for 60 days. GC-TOFMS was utilized to analyze oxygenated compounds and biomarkers. The conclusions of this study are summarized as follow:

1. GC-TOFMS was used to analyze the distribution of oxygenated compounds, which may contain sulfur, and/or nitrogen. The peaks of the oxygenated compounds can be distinguished from selected mass to charge (m/z 73). The identified compounds include $C_{17}H_{34}O_2$, $C_{19}H_{36}O_2$, $C_{22}H_{34}O_4$, $C_{26}H_{50}O_4$, and $C_{27}H_{42}O_4$.
2. The ratio of acyclic to cyclic (A/C) naphthenic acid of fresh crude oil is >1 , indicating that the acyclic naphthenic acid compound is more abundant than cyclic naphthenic acid. However, the naphthenic acid compounds are not resistant to the weathering process due to biodegradation, photo-oxidation, evaporation, and dissolution.
3. The A/C ratio of fresh crude oil samples is between 1.02 to 3.20, while the fresh fuel oil is 0.58 and 0.98. In general, weathered crude oil samples have a ratio of 0.67 to 2.59, except for Kissanje crude oil on day 60, which has a ratio of 0.47. The A/C ratio of weathered fuel oil is <0.65 . Moreover, the A/C ratio can be considered supporting information to differentiate crude oil and fuel oil in this research since fuel oil exhibits a lower A/C ratio than crude oil.
4. The distribution of the hopane group is useful for oil fingerprinting. The distributions of hopane groups from the same crude oil region seem to have a similar pattern. In contrast, the different crude oil origin regions have different hopane distribution patterns.

5. The diagnostic ratio of selected ion m/z 191 is reliable and provides an accurate correlation plot between day 0 and day 30. This technique is suitable for oil fingerprinting due to its low error value because of three times repetition of sample analysis. Moreover, the error values were calculated with a 95% confidence level interval.

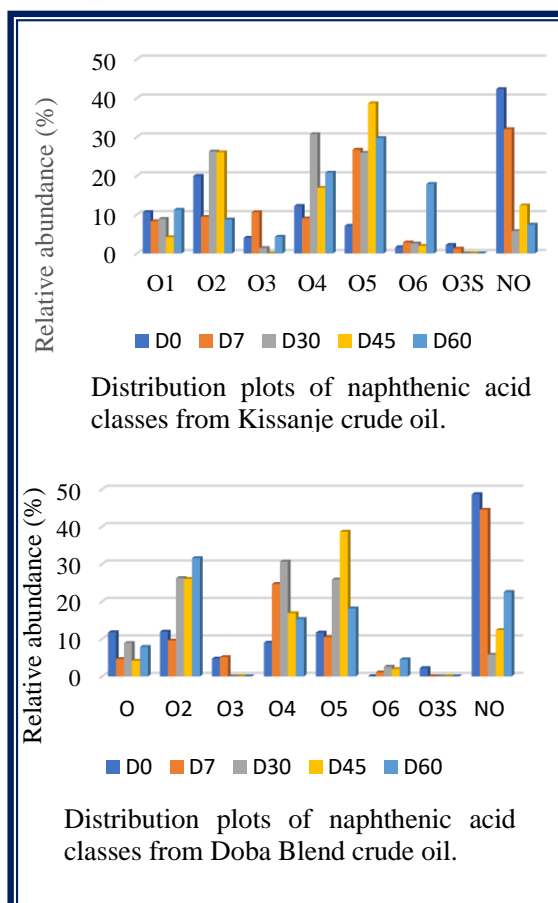
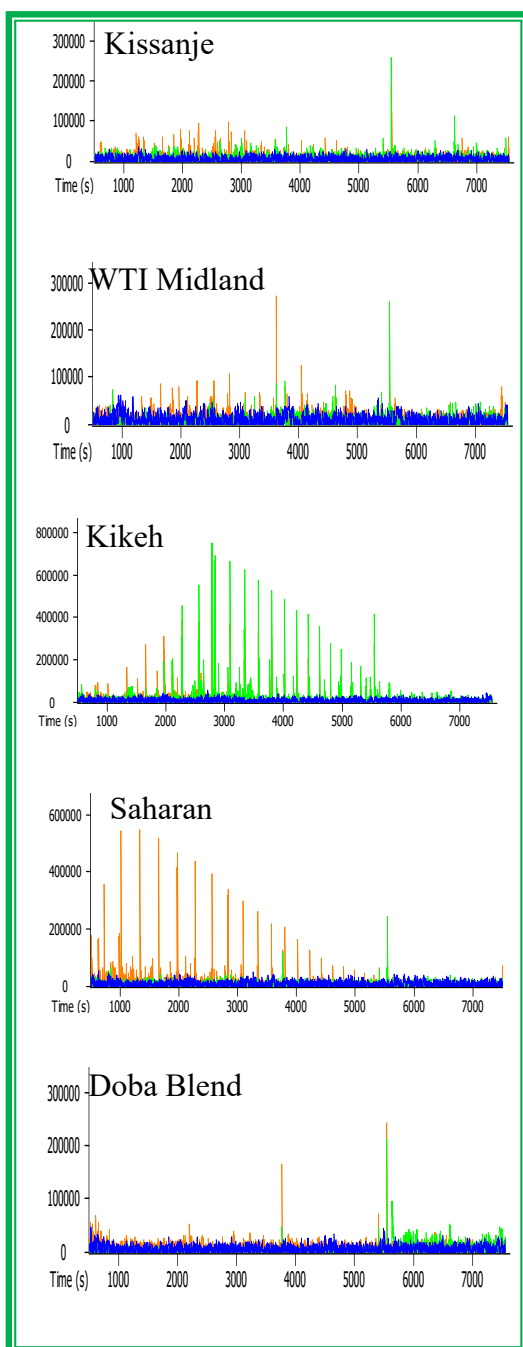
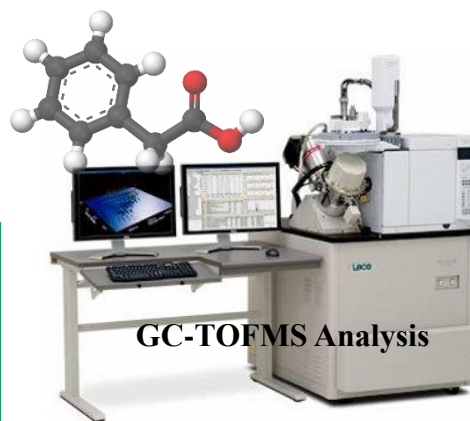
5.2 Recommendations

The identification of oxygenated compounds, especially individual naphthenic acid compounds, is still challenging in environmental studies because it is a complex mixture after it is released into the environment. The naphthenic acid extraction techniques for oil spill identification need to be further studied.

APPENDIX GRAPHICAL ABSTRACT

Appendix A

Oxygenated Compounds Distribution in Crude oil



The oxygenated compounds are not resistant to the weathering process due to biodegradation, photo-oxidation, evaporation, and dissolution.

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