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Utility of Biomarkers in Crude Oil Identification and Forensic Tracking of Spilled Oil

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ABSTRACT

This paper reviews the use of forensic fingerprints of molecular biomarkers for tracking the source of spilled oil. Crude oil is a natural organic substance found in oil reservoirs and seep, that undergoes natural degradation process which alters its chemical composition when exposed to environmental conditions. Biodegradation of crude oil occurs because eubacteria and archaea microorganisms have evolved specific pathways and mechanisms that enable them to digest hydrocarbons in crude oil. Biomarkers are one among several groups of hydrocarbons found in crude oil, they exist in low concentrations and are detected by gas chromatographic-mass spectrometric analysis. Biomarkers such as hopane and steranes which are comparatively resistant to biodegradation are used as internal standards to assess the extent of oil weathering, oil mass loss and percentage oil depletion during weathering. During forensic oil spill investigations, biomarker fingerprints provide critical information used for tracking the source of oil spill; evaluate extent of oil degradation and compositional alterations in weathered oil under different environmental conditions. In oil spill forensic investigations, biomarker fingerprints provide information such as chromatographic profiles, distribution patterns and diagnostic ratios, which are widely used for correlation and differentiation and in oil-source attribution, as well as to assess the fate, behavior and the effects of spilled oil on the environment.

Keywords: Hydrocarbons; Biomarkers; Oil spill, Forensic identification; Fingerprinting; Dispute resolution

Introduction

Crude oil is a natural occurring substance which is composed of complex mixture of a variety of hydrocarbons formed from remains of dead living organisms, which have over a long period of time undergone different biogeochemical changes while buried under the earth[1,2]. Over the years crude oil has become a natural product of immense economic importance which can be refined into various fuel products and its bye-products are feedstock for production of petrochemicals and several other industrial materials[3]. Refined fuel products are beneficial to people as energy sources to operate automobile and industrial

equipment which improve industrialization, global economies and public wellbeing[4]. Petrochemicals from crude oil are used as raw material feedstock for production of a variety of other useful industrial chemicals, goods and services. Globally, crude oil is an economic resource that influences the political and economic stability of a country because it is crucial in economic planning, security and very fundamental in economic decision-making[5,6]. Crude oil is the major economic resource of some oil-producing countries and is a critical economic factor that determines economic health, social and political stability and national security of these countries[7-9].

Effects of oil spills

Factors such as global population growth, migration to industrialized cities, imbalances in global oil distributions and strategies to balance global oil demand and supply have resulted in environmental oil spills[10,11]. Oil spills occur when there is deliberate or inadvertent release of toxic hydrocarbons from crude oil into the environment through uncontrolled production; transportation and use of petroleum and its refined products[12]. When oil spills on aquatic environments, it spreads along with winds and ocean currents into beaches and results in widespread oil-slicks with consequential lethal impacts on marine organisms and ecosystems[13]. Oil slicks disrupt photosynthesis, reduce dissolved oxygen and compromise the temperature of sea elements in the affected ecosystems[14]. Oil slicks stick on feathers and furs of marine aquatic organisms, undermine breathing in these organisms and often results in mortality of these organisms[15]. Spills in coastal wetlands and mangrove ecosystems adversely impact those environments and disrupt biodiversity in such ecosystems[16]. Oil spills in terrestrial environments are widely caused by pipelines bursts, leaks from underground and surface storage tanks and spillages from oil transportation tankers and trucks. Oil spills on terrestrial ecosystems provoke direct inhalation of toxic hydrocarbons by humans and organisms[17]. They disrupt ecological processes and loss of natural resources, decrease economic activities and loss of means of sustenance, which ultimately leads to community decline[18]. Persistent hydrocarbons from spilled oil on land could migrate through aquifer to contaminate ground water and might result in DNA degradation, compromise cellular immunity and instigate growth of cancer cells in organisms[19].

Biomarkers in crude oil

Based on hydrocarbon composition, crude oil consists of several groups of hydrocarbons and their isomers, including normal and cycloalkanes, simple aromatics, alkylated polyaromatic hydrocarbons (PAHs) and biomarkers[20]. These hydrocarbon groups are commonly used for oil spill identification, source correlations and forensic characterization[21]. Biomarkers are complex molecular fossils in crude oil, that are derived from biological precursor compounds of dead living organisms such as terpenoids, sterols and steroids[22]. They preserve chemical and structural features of their parent biological organisms and are widely used to provide critical information regarding organic matter sources, crude oil depositional environment, its thermal maturity and biodegradation history[23-25]. Biomarkers are biodegradation-resistant and have similar molecular structures to their biological precursors. These characteristics distinguish biomarkers from other crude oil hydrocarbons and make them critical diagnostic indicators in forensic oil spill investigations[26,27]. Biomarkers are critical components of forensic oil spill investigation, they are essential indicators for source identification and differentiation of spilled oil from potential source oil[28,29]. **(Figure 1)** illustrates the structural similarities between hopanes in sediments and bacteriohopanestetrol of prokaryotic organisms, while **(Figure 2)** shows the diagenetic pathway of sterols in sediments which leads to the formation of steranes in crude oil.

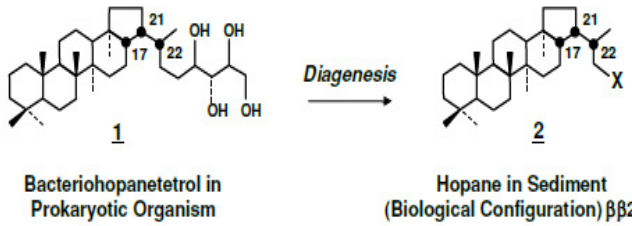

Figure 1: Structural similarities between hopanes in sediments and bacteriohopanestetrol of Prokaryotic organisms[30]

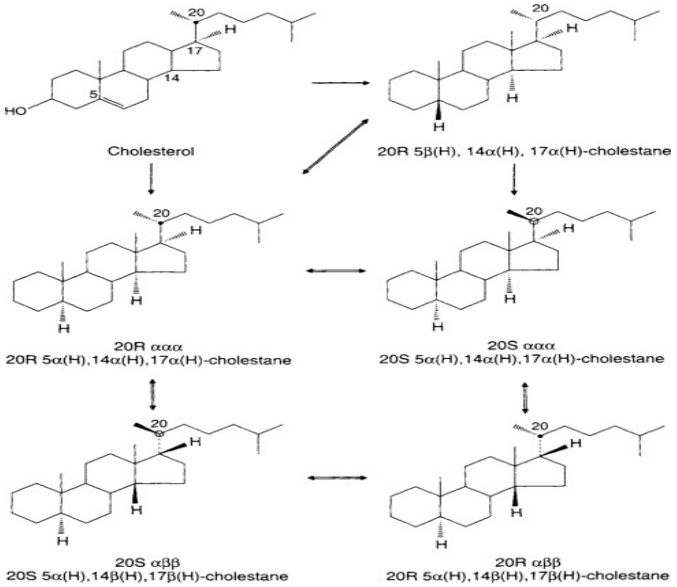

Figure 2: Diagenesis of sterols in sediments which leads to steranes in crude oil[31]

Triaromatic steranes are high molecular weight biomarkers that are relatively resistant to biodegradation and used to track sources of biodegraded oil. In addition, their fingerprint distributions are used in assessment of organic matter maturation, evaluation of extent of spilled oil weathering and environmental mitigation strategies and in oil-source rock correlations[32,33]. Diasterane and cholestane are crude oil biomarkers which originated during diagenesis stage of sterol formation from living organisms are commonly used in assessing weathering resistance and hydrocarbon persistence in the environment. The fingerprints of these biomarkers are used as evidence in legal forensics and oil spill forensic admissibility[34,35].

Hopanoid are biomarkers that are used as geochemical indicators in oil-source correlations to determine depositional source rocks, depositional conditions, source lithology and maturity of the hydrocarbons[35-37]. Polycyclic aromatic hydrocarbon biomarkers are commonly applied in crude oil geochemistry and environmental forensic investigations for assessment of spilled oil weathering[38], to distinguish between terrestrial and marine organic input, for oil and sediment determination during oil-source geochemical correlations[39,40]. Bicyclic sesquiterpenes are another group of aliphatic biomarkers that are distributed widely in crude oils and source

rocks. Their fingerprints are used as diagnostic indicators for oil spills caused by fuel oil and refine oil products, they are applied as indicators of biodegradations to explore the genesis of organic materials[41,42].

Biomarkers are critical repositors of geochemical information in oil-source correlation studies. They provide insight into nature and type of original organic matter, depositional environment, geological conditions during organic source formation, thermal maturity and geologic age of the source rock and oil[30,37,43]. In oil-oil and oil-source rock correlation investigations, distribution profiles and diagnostic ratios of biomarkers are critical parameters for understanding reservoir interconnectivity and hydrocarbon migration pathways. Biomarkers provide geochemical information necessary for evaluation of crude oil distributions and production patterns with sedimentary basins[1,30,44].

Dahl, et al.[45] reported that biomarker distributions in crude oil seeps can be used to indirectly infer the quality and characteristics of source rocks in situations where the source rock samples are unavailable. Volkman[46], established that compositions of biomarkers in crude oil and rock extracts are mainly controlled by environmental depositional conditions and the source organic matter input. Jiang & Fowler[47], reported that under favourable depositional conditions, diagnostic precursors of biomarkers are produced by relatively large community of a few organisms. For example, the abundance of β-carotene in aquatic bottom sediments suggest organic matter input derived from halophilic bacteria[30]. Also, the distributions and relative abundances of C_{27}, C_{28} and C_{29} steranes in crude oils are indicative of organic matter input derived from eukaryotic organisms deposited in sediments from overlying water column[2,48,49]. Scholars reported that the diasterane/sterane ratio in crude oil reflects the relative abundance of clay minerals in the source rock, while as a high abundance of dinosterols in sediments is indicative of dinoflagellate blooms in nutrient-rich marine waters[50,51].

Biomarker-Based Oil-Source Correlations

Crude oils derived from different source rocks contain hydrocarbons with different range of carbon atoms; they exhibit distinct biomarker distributions and show characteristic relative differences in their biomarker compositions[22]. Therefore, comparing the qualitative distribution patterns of biomarkers in crude oils is essential in establishing reliable oil-source correlations. Oil-source geochemical correlations using biomarkers involve several key steps. First determine whether the target biomarkers are present within a defined carbon number range in both the oil and the suspected source oil/rock extract. Second, a detailed evaluation of biomarker profiles and qualitative distribution patterns is conducted to assess similarities between the oil and the proposed source. In addition, diagnostic ratios of selected target biomarkers are compared to determine the degree of similarity. Finally, another analysis is undertaken to identify any distinct or unique biomarkers that may differentiate the oil from the suspected source, thereby strengthening or refuting the proposed correlation[2,22,30]. Positive match in biomarker distribution patterns between oil and suspected source oil/source rock extract may suggest a genetic relationship; it does not constitute conclusive proof that they originated from the same source. Such similarities may indicate a possible correlation; conclusive inferences must be supported with additional geochemical evidence. Conversely, significant disparities in biomarker distributions between the oil and the proposed oil/rock source may indicate a lack of correlation[2,30].

Shen, reported that n-alkane compositional alterations in weathered oil pose a challenge in use of n-alkanes as source indicators for oil spill identification. They demonstrated that application of biodegradation resident biomarkers such as steranes and hopanes can be used to minimise weathering interference thereby allowing for more reliable oil-source identification. Volkman, et al.[52] investigated distributions of biomarker such as steranes, hopanes and triterpanes, in hydrocarbon-contaminated aquatic sediments from estuarine and coastal regions of Australia. They demonstrated that lubricating oil was one of the probable anthropogenic sources of pollution. Currie, et al. investigated the fingerprints and distribution profiles of triterpane biomarkers in tar balls collected from Western Australia Coast and reported that the oil spill contamination originated from Southeast Asian crude oil.

Barakat, et al.[53] analyzed distribution patterns of five crude oil samples from Gulf of Suez using GC-MS, on evaluation of crude oil quantitative biomarker parameters, the five crude oils were classified into three genetically distinct oil types based on differences in source characteristics and depositional environment. Wang, et al.[12] investigated a 1998 oil spill at a factory in Acton Vale, Quebec, using GC–FID and GC–MS techniques. Although the GC–FID chromatogram of the weathered oil differed from that of the suspected source oil due to weathering, the distribution patterns of terpane, sterane, C_{21}-C_{35} triterpanes and C_{30} βα-hopane biomarkers were essentially identical. These results confirmed that the spilled oil originated from a Bunker C fuel oil obtained from the factory heat exchanger. Wang, et al.[21] characterized three petroleum samples from Montreal using gas chromatographic techniques. Although GC–FID chromatograms identified all three samples as hydraulic fluid products with similar bulk profiles. However, detailed analysis of biomarker distribution patterns and diagnostic ratios revealed that two samples were essentially identical, whereas the third sample exhibited distinct biomarker characteristics. Seifert & Moldowan[39], indicated that although steranes and triterpanes are good biomarker indicators for oil-source correlations investigations, their reliability can be limited in highly biodegraded oils and in severe biodegradation conditions. Oliveira, et al.[54] reported that polycyclic aromatic hydrocarbons (PAHs) and sulfur-containing aromatic compounds such as thiophenes and dibenzothiophenes are commonly employed for oil-source correlations involving advanced biodegradation or severely biodegraded oil.

Crude oil and fingerprinting techniques

Crude oil is a natural organic substance characterized by complex and variable chemical compositions which differ from one crude oil to another[30]. Generally, each crude oil possesses a unique intrinsic chemical fingerprint used to differentiate it from others and such intrinsic characteristics can be used in spill source identification, oil-oil correlation and forensic oil-spill investigations[55]. Fingerprinting technique is one of the major geochemical analyses used to understand chemical characteristics of crude oil, determine the fate and behaviour of spilled oil and is very crucial when addressing environmental oil spill-related problems[56].

Biomarker fingerprinting is essential in identifying spilled oil biomarker distribution profiles which are used to establish the source of the crude oil contamination during environmental forensic investigations[57]. In cases involving multiple spill sources from different responsible parties, oil spill forensic fingerprinting is essential for oil-source apportionment, contamination assessment and in the evaluation of legal liabilities among contributory spillers[58]. Forensic fingerprinting analysis frequently serves as critical scientific evidence in enforcement of oil pollution regulations, guidelines and policies. It is used to allocate liability among responsible parties, determine compensation for affected stakeholders and support the assessment of cleanup and remediation costs[59,60].

The complex hydrocarbon compositional nature of crude oil and the low concentrations of specific crude oil hydrocarbons such as biomarkers, can present significant challenges to the application of fingerprinting techniques in forensic oil spill investigations[57,61]. To overcome these analytical challenges, accurate and reliable fingerprinting techniques such as chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) are essential for characterizing hydrocarbon distributions in spilled and source crude oil samples[59].Therefore, the assessment of diagnostic ratios of source-specific hydrocarbons, including isoprenoids, alkylated polycyclic aromatic hydrocarbons (PAHs) and biomarkers, is widely used to identify and correlate spilled oils with their potential source crude oils[62].

Fingerprinting techniques and oil spill dispute resolutions

Oil pollution impacts on the health of organisms, adversely affects natural resources and depreciates economic assets and reduces the aesthetics value of impacted ecosystems. Therefore, accurate assessment of hydrocarbon contaminants and reliable oil-spill source identification approach are critical components of environmental investigations and oil spill liability litigation[19,33]. To minimize the incidence and severity of environmental oil spills, effective enforcement of oil-spill prevention and mitigation laws, regulations and policies is essential, alongside mechanisms that ensure responsible parties are held accountable for victim compensation and environmental remediation costs[61].

In course of performing their activities, oil producing and services companies in some instances attempt to misrepresent deliberate or inadvertent oil spill incidents, probably to mitigate reputation damage and to avoid punitive measures such as litigation, penalties and compensatory liabilities. Such actions often lead to culpability disputes and may complicate conflict resolution processes between operating companies, as well as between operators and regulatory authorities[33]. To resolve oil spill disputes, determine liabilities and assess damages attributable to responsible parties, forensic chemical characterization of spilled oil is essential for defensible source identification and correlation to a suspected source crude oil[59]. In addition, definitive characterization and accurate source identification of oil spills are essential for predicting potential long-term ecological impacts and resolving disputes related to oil pollution liability and compensation[56].

Forensic oil spill fingerprinting is important because it provides scientific evidence necessary to assess economic losses and environmental damage resulting from oil pollution[63]. It generates comprehensive hydrocarbon fingerprinting data which, when integrated with environmental, geological, historical and other geochemical information, can serve as a defensible basis for determining legal responsibility and supporting litigation proceedings related to oil spill incidents[64]. Oil spill fingerprinting techniques are used for defensible oil characterization during dispute resolution processes and for source identification by correlating spilled oil fingerprints with multiple suspected source oils[65-67]. Data derived from oil spill fingerprinting analyses are used to distinguish crude oil hydrocarbons from background hydrocarbons of biogenic, pyrogenic and petrogenic origins[68,69]. Fingerprinting analysis is commonly applied in forensic oil spill investigations to monitor compositional alterations resulting from weathering and to provide supporting evidence used for evaluation of oil spill victims' compensatory claims[70-73].

Oil Spill Identification Protocols

Oil spill identification protocols comprise of collections of scientific procedures and analytical methodologies used to identify and characterize oil spill incidents and to trace spilled oil back to its source. These protocols usually integrate forensic chemical fingerprinting techniques-such as gas chromatography (GC-FID), gas chromatography-mass spectrometry (GC-MS), other analytical methods with biomarker analysis to establish source-spill correlations[29,33,74,75]. Oil spill identification protocols encompass a collection of scientific systematic procedures, analytical methods and techniques employed in determination of hydrocarbon distribution patterns and oil profile characterization. These protocols widely involve laboratory analysis of using GC-FID and GC-MS techniques used for assessment of chromatographic fingerprints, diagnostic ratios of source-specific alkane, isoprenoids, polycyclic aromatic hydrocarbons and biomarkers in crude oil. Application of multivariate statistical analysis on calculated diagnostic ratios enables defensible comparisons and correlations between spilled oil and potential source oil which results in reliable source oil attribution[76,77].

Integrated Multivariate Oil Fingerprinting (IMOF)

The IMOF oil spill identification protocol is a new approach that is designed to facilitate fast and accurate source identification. It applies comprehensive multivariate statistical analysis to hydrocarbon fingerprint data obtained from spilled crude oils, refined petroleum products and potential source oil samples. By systematically analysing these datasets, the protocol enables defensible correlation between the spilled oil and its suspected source[29,33,59].

The protocol combines hydrocarbon fingerprinting data from several chromatographic and spectrometric techniques such as gas chromatography-mass spectrometry (GC-MS) and fluorescence spectroscopy with multivariate statistical analysis. By combining these analytical methodologies, the protocol enables detection of subtle chemical compositional variations in spilled oils that may result from environmental weathering processes. The application of multivariate statistical approaches, such as principal component analysis (PCA) and other statistical tools, commonly facilitates objective interpretation of complex fingerprint data by reducing subjectivity in visual pattern recognition and enabling statistical classification of samples. Therefore, this approach allows researchers to distinguish compositional alterations caused by weathering from characteristic differences between oil sources, in that way improving the defensibility of

oil–source correlations[30,33]. IMOF approach enhances forensic interpretations in challenging scenarios particularly in moderate to advance stages of oil weathering which leads to alterations in chemical compositions of the spilled oil. It incorporates data preprocessing and visualization procedures that facilitate the assessment of weathering effects[76]. Before the application of statistical analysis on the fingerprint data, the protocol employs fingerprint chromatographic preprocessing steps such as baseline correction, chromatogram alignment and normalization to reduce analytical noise and to isolate compositional changes due to oil weathering. In addition, the IMOF methodology, as seen in **(Figure 3)**, evaluates the entire fingerprint profile and selected diagnostic ions in the samples, unlike conventional spill identification approaches, which rely on limited diagnostic ratios. This protocol enables more effective tracking of chemical compositional shifts and alterations associated with oil weathering and improves the reliability of oil–source correlation[59,78].

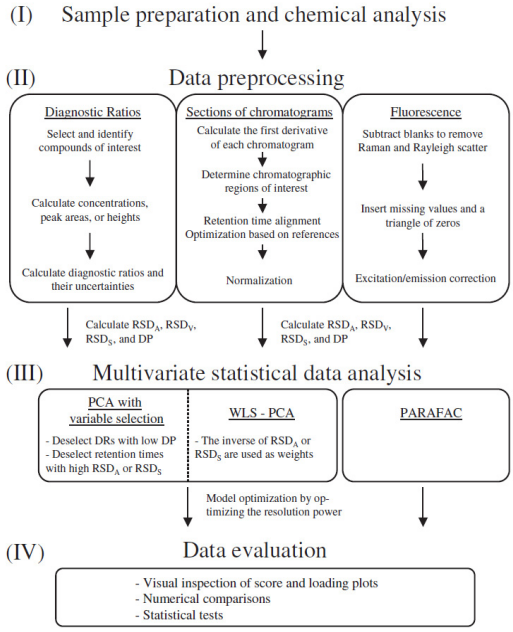

Figure 3: A schematic diagram of Integrated Multivariate Oil Fingerprinting (IMOF) oil spill identification protocol[59]

European Committee for Standardization (EN/CEN/TR 15522)

The European Committee for Standardization (CEN) established standardized analytical procedures and methodologies for the emergency forensic investigation of oil spill incidents[79-81]. These protocols involve the application of analytical techniques for chemical evaluation, chemical characterization and identification of spilled oil and related environmental samples. The protocol combines oil spill identification procedures with multivariate statistical analysis to assess similarities and differences in chromatographic profiles and biomarker ratios between oil spill samples and their potential source oils[82]. Oil spill chemical evaluation assesses impact and environmental risk associated with oil pollution, estimates the oil had weathered and establishes geochemical correlations between spilled oil and potential source oil. CEN EN 15522 approach to environmental forensic investigations is established to improve reliability and defensibility of oil-source attribution. CEN forensic oil spill identification methodology commonly involves oil extraction with solvents to remove impurities, spilled oil and source oil hydrocarbon chemical characterization using gas chromatographic techniques. Geochemical correlations and source attribution are established by comparing chromatographic profiles, hydrocarbon distribution patterns and diagnostic ratios of specific biomarkers in spilled oil and potential source oils[56,82,83].

Tiered analysis and data treatment

Tiered analysis and data treatment protocol is an oil spill identification methodology that is based on the revised Nord Test method and widely used for environmental oil spill forensic investigations[28,79,84]. Identification process for geochemical attribution between spilled oil and source oils is categorized into three levels of experimental analysis and data treatment.

First level of tiered analysis involves sample preparation, hydrocarbon group determination, distribution patterns and product screening using GC-FID to characterize and determine whole crude hydrocarbon compositions from C10-C40. Visual preliminary screening of fingerprints of spilled oil and potential source oils to characterize overall carbon distribution and profiles of hydrocarbons from C10-C40. Comparative assessment of fingerprints and selected isoprenoid ratios are commonly used to evaluate weathering checks and objectively compare environmental samples to establish similarity at the first level of analysis. Samples that are not similar or nonmatch are eliminated from further analysis. Uncertain samples are used for further identification processes/additional level of analysis. The level provides initial screening assessment that determines further investigations in next tier levels of oil spill forensic analysis[28,81].

Second level of the tiered analysis involves biomarker fingerprint analysis on samples using GC-MS technique in selected ion monitoring mode for fingerprint data and matching distribution patterns of target PAHs and specific biomarkers. This analytical level focuses on generating fingerprint data for comparison of the distribution patterns of target polycyclic aromatic hydrocarbons (PAHs) and specific petroleum biomarkers. Semiquantitative data obtained from these analyses are used to calculate diagnostic ratios of selected PAHs and biomarkers, including hopanes, triterpanes, steranes, diasteranes and triaromatic steroids. These biomarker ratios are highly resistant to weathering and biodegradation and therefore provide reliable molecular indicators for correlating spilled oil with potential source oils. Comparative evaluation of diagnostic ratios and biomarker distribution patterns enables more definitive source attribution than the preliminary screening performed in Level 1 analyses[80,84].

The level 3 tiered of the tiered level approach focuses on detailed assessment and analytical comparison of source-specific biomarkers between spilled oil, potential source oil samples and other established fingerprinting databases and literatures. Fingerprinting information from this tier level pf analysis is critical for oil weathering assessment, evaluation of semiquantitative distribution profiles and diagnostic ratios of PAHs groups and biomarkers. Diagnostic ratios with considerable variability are evaluated and eliminated, while accurate diagnostic ratios are utilized for oil-source correlations[28]. The third level of tiered analysis provides more reliability, better defensibility of oil-source attribution and supports interpretations of oil spill identification results[81]. This is lucidly illustrated by the schematics in **(Figure 4)**.

Figure 4: Tiered analysis and data treatment oil spill identification methodology.

Crude oil Weathering and Biodegradation

Crude oil is a natural occurring hydrocarbon substance, which when exposed to the environment by way of oil spillage usually undergoes physical, biological and chemical weathering processes that alter its chemical compositions[85,86]. Through evaporation and biodegradation processes, oil weathering causes loss of light, less recalcitrant and low molecular weight hydrocarbons from spilled crude oils[87,88]. Weathered crude oil retains certain hydrocarbons such as isoprenoids, PAHs and biomarkers that are used for oil spill forensic characterization and identification to link spilled oils to their source oils through forensic fingerprinting techniques[21]. When oil spills, environmental factors such as evaporation, dispersion, emulsification, oil-sediment aggregation, sedimentation, dissolution, photooxidation and microbial degradation influence the behaviour of spilled oil, its ecological effects on organisms and its long-term fate on the environment[64].

The degree of oil compositional alteration when crude oil is exposed to the environment is influenced by environmental conditions and the nature of residence microorganisms present in the spill site[88]. Chemical alterations of crude oils through weathering processes take place in several scenarios including oil depositional environments, during sedimentation process in oil storage tanks and vessels and in oil spillages[89,90]. Weathering mechanism for oil degradation is determined by factors such as type of crude oil, physicochemical characteristics of the oil and other predominant physical, chemical and geologic factors around the oil spill site[91,92]. During spilled oil weathering process, the degree of modification of hydrocarbon molecular structure and the formation of the weathered products serve as geochemical indication of the deterioration, degradation stage, weathering extent as well as the residence time of the spilled oil[22]. Mechanisms for crude oil weathering involve a chemical redox reaction process such as cracking, metabolization and free radical formation and other biochemical degradation reactions that alter molecular structures and compositions of biomarkers and other crude oil hydrocarbons[30,93,94]. Oil weathering assessments and geochemical indices used to evaluate extend of weathering in oil are derived from known principles of petroleum microbiology, kinetics of hydrocarbon degradation and physicochemical transformation mechanisms of hydrocarbons[95-97]. **(Figure 5)** describes the biochemical reactions that take place in the crude oil biomarker weathering process.

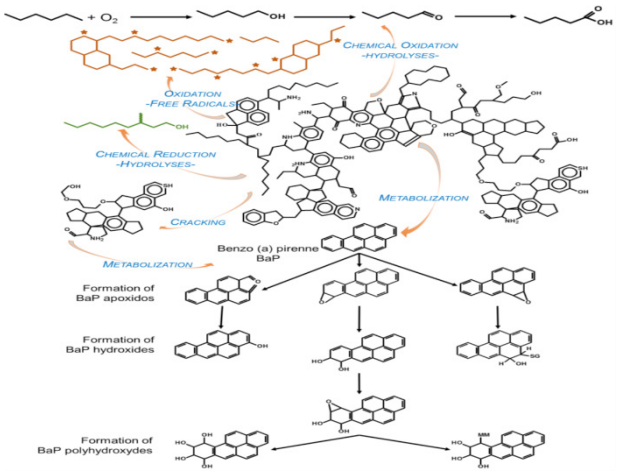

Figure 5: Biochemical reactions in crude oil biomarker weathering process[98].

Oil weathering assessment

Assessment of the degree of oil weathering, which evaluates the percentage depletion of crude oil exposed to environmental conditions, is commonly determined using diagnostic ratios of selected isoprenoids and biomarker compounds[30,99]. Diagnostic ratios commonly used to evaluate the degree of oil weathering include pristane/phytane, pristane/n-C_{17}, phytane/n-C_{18}, C_{30} hopane/gammacerane, relative abundances of C_{27}–C_{29} steranes, Ts/Tm, C_{29} sterane isomer ratios and the 22R/22S homohopane ratio[100-102]. Oil spill fingerprint data are incorporated with quantitative models and biomarker diagnostic calculations for estimation of extent of crude oil degradation over a given period and for interpreting oil environmental weathering processes[22,59,103-105]. Quantitative models used for estimating degree of oil weathering are based on concept which involves preferential degradation of certain hydrocarbons and chemical alterations of spilled oil compositions. As biodegradation progress with time and severity, there is a relative increase in concentrations of biodegradation resistant compounds such as hopanes and steranes[22,106,107,108].

Terpane hopanoids such as C_{29} 17α(H),21β(H)-norhopan$_e$ and C_{30} 17α(H),21β(H)-hopane have been extensively used as conserved internal biomarkers to estimate the extent of spilled oil weathering[94,109]. The degree of oil spill weathering is determined by comparing the concentration of conserved hopane biomarkers in the weathered oil with their corresponding concentrations in the source oil[30,110,111]. In oil spill forensic investigations, the extent of oil weathering can be calculated using data obtained from GC-MS analysis either by comparing conserved hopane concentrations in the weathered oil and source oil or by assessing the depletion of individual target analytes using Equations (1), (2) and (3), respectively[22,30,33]. *Equation (1) is used to calculate the overall percentage of oil depletion. Equation (2) depletion of a specific biomarker (individual analytes) in weathered crude oil and corrects for overall. Equation (3) is utilized to determine the relative change in abundance of a target biomarker (individual analyte) normalized to hopane in weathered crude oil over time.*

Oil depletion (%) = (1-A0/A1) *100 ..... (1)

Where A1 represents hopane concentration in the weathered

oil and A0 represents the concentration of hopane in the source oil[112].

Analyte depletion (%) = [1- (B1/B0) *(A0/A1)] ..... (2)

Where B1 represents the concentration of the target biomarker (analyte) in the weathered oil and B0 represents its concentration in the source oil. Similarly, A1 and A0 denote the concentrations of hopane in the weathered oil and source oil, respectively[113].

Percentage Depletion = [(B0/H0) - (Bt/At)/(B0/A0] * 100… (3)

Where B0 represents the initial concentration of the target biomarker and A0 represents the initial concentration of C_{30} hopane at time zero. Similarly, Bt and At, denote the concentrations of the target biomarker and C_{30} hopane, respectively, at a given time[113].

There is limitation in the choice of hopane as a reference biomarker for calculating oil mass loss during oil weathering because of low hopane concentrations in some light crude oils and refined petroleum products[12,94]. In cases where tricyclic and pentacyclic hopanes occur at low concentrations or are entirely not present in the fingerprint; bicyclic sesquiterpene biomarkers characterized by unique molecular structures, strong chemical stability and high resistance to weathering can be used as alternative reference compounds in oil spill investigations[20,114,115]. In their oil spill forensic investigations, Wang, et al., employed C_{15} and C_{16} sesquiterpenes as internal reference compounds to estimate the extent of weathering in lighter, diesel-type fuels. Similarly, Using sesquiterpenes as reference biomarkers, while investigation evaporation weathering in diesel and three moderated weathered crude oil samples. Yang, et al.[20] demonstrated that oil mass loss calculations obtained by using sesquiterpenes reference biomarker compares closely with weathering percentage calculations obtained by using C_{30} αβ-hopane reference.

Another biomarker ratio commonly used to estimate the extent of oil weathering is the Ts/Tm (Ts (18α(H)-22,29,30-trinorhopane) and Tm (17α(H)-22,29,30-trinorhopane) index, which represents the ratio of two isomeric peaks of the C27H46 trinorhopane biomarker. This index is frequently employed for assessing organic matter input and the maturity of crude oil sources[30,37]. Equation (4) represents the Ts/Tm index, which is commonly used to estimate the extent of the weathering process in crude oils[100,116].

Ts/Tm index = Ts / (Ts +Tm) ..... (4)

Where Ts represents the concentration of 18α(H)-22,29,30-trinorhopane and Tm represents the concentration of 17α(H)-22,29,30-trinorhopane in the weathered oil. Considering that the Tm isomer is more susceptible to biodegradation than the Ts isomer, an increase in the Ts/Tm index commonly indicates progression of the weathering process[30].

Sterane ratios are useful diagnostic parameters for assessing the degree of oil weathering due to their relative resistance to biodegradation and the stability of their compositional proportions during early stages of weathering[22,100].The relative percentage abundance of C_{27}, C_{28} and C_{29}-sterane biomarkers in crude oil are also commonly used for evaluating crude oil maturity indices and estimating extent of weathering by substituting each sterane group in mass balance or depletion equations below[100].

$$\%C_{27}\text{-sterane} = [C_{27}/ (C_{27} +C_{28} +C_{29})] *100 \quad \text{..... (5)}$$

$$\%C_{28}\text{-sterane} = [C_{28}/ (C_{27} +C_{28} +C_{29})] *100 \quad \text{..... (6)}$$

$$\%C_{29}\text{-sterane} = [C_{29}/ (C_{27} +C_{28} +C_{29})] *100 \quad \text{..... (7)}$$

Where C_{27}, C_{28} and C_{29} present the concentrations of the C_{27}, C_{28} and C_{29}-steranes in the weathered crude oil.

C_{29} steranes are important biomarkers used for evaluation of crude oil source input, depositional environment and thermal maturity. C_{29} steranes have stereoisomers such as 20S/20R and ββ/αα ratios whose distribution in crude oils commonly reflects progressive isomerization towards thermodynamic equilibrium **(Figure 6)**. In oils spill forensic investigation, the distribution patterns of these stereoisomers and their relative resistance to biodegradation are critical parameters for estimation of oil degradation extent in moderately and severely weathered oils[30,116].

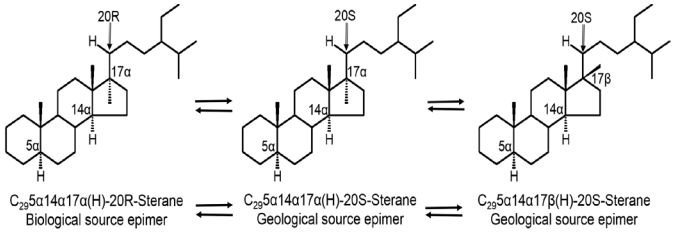

Figure 6: Stereoisomers of C_{29} steranes.

Crude oil biodegradation mechanism

Biodegradation of hydrocarbons in crude oil occurs because some microorganisms such as eubacteria and archaea have evolved specific metabolic pathways and mechanisms that aid them to degrade saturated and aromatic hydrocarbons in crude oils[30,117]. Biodegradation of crude oil differs from the evaporation weathering process of crude oil in several ways; while evaporative weathering involves loss of volatile and low molecular weight hydrocarbons from the crude oil due to vaporization, biodegradation involves selective breakdown of hydrocarbons through microbial biochemical transformation processes[2,106].

Different hydrocarbon groups in crude oil exhibit diverse vulnerabilities to microbial degradation activities[27,118].The vulnerability of crude oil hydrocarbons to microbial degradation is influenced by multiple factors including characteristics of the environment, nature and activity of the microbial community, availability of nutrients, presence of microbial toxic contaminants, intrinsic physical and chemical properties of the crude oil, as well as molecular structure and size of hydrocarbon[115]. Biodegradation of hydrocarbons decreases with increasing molecular weight, degree of branching, extent of cyclization and number of heteroatoms within the compound[119]. It follows a preferential sequence in which n-alkanes are degraded first, followed by branched alkanes, simple and low molecular weight aromatics and finally high molecular weight aromatic compounds (PAHs)[93,30,117]. N-alkanes degradation proceeds through sequential cleavage and oxidation of terminal carbon–carbon bonds in saturated hydrocarbons. While as, branched and cyclic saturated hydrocarbons are degraded more slowly because of the existence of tertiary and quaternary carbon atoms, which introduce steric hindrance and structural stability that indices resistance to microbial attacks[43].

Whittaker & Pollard[120], reported that straight chain alkanes are more susceptible to microbial degradation than aromatic hydrocarbons, which in turn are more vulnerable than resins and highly condensed asphaltenes. Killops and Killops[43], demonstrated that chromatographically resolved hydrocarbons in crude oil are often preferentially degraded compared to unresolved complex mixtures components. Head, et al.[93] reported that preferential degradation of low–molecular weight saturated aliphatic is usually interpreted as evidence of early stages of oil biodegradation. Empirical studies have demonstrated that crude oil hydrocarbons are biodegraded in a generally decreasing order of susceptibility as follows: n-alkanes > benzene > toluene > isoalkanes and anteisoalkanes > cyclohexylalkanes and methylcyclopentylalkanes > acyclic isoprenoids >> naphthalene > phenanthrene >> higher molecular weight polycyclic aromatic hydrocarbons (PAHs) > C27–C29 steranes > C30–C35 hopanes > diasteranes > C27–C29 hopanes > C21–C22 steranes > tricyclic terpanes[30,108,121,122].

The trend is a selective hydrocarbon removal process, which involves a preferential and faster degradation of certain hydrocarbons more than the others and not a compete removal of one hydrocarbon before the next in the trend. Moderate biodegradation level is commonly indicated by near complete removal of n-alkanes and partial alterations in compositions of certain other hydrocarbons including cycloalkanes, alkylbenzenes and acyclic isoprenoids, indicates moderate level of biodegradation[115]. Advance stage of bioregulation is suggested by strong degradation of isoprenoids, aromatics such as alkylbenzenes, naphthalenes, slight and trong depletions of terpanes and steranes, formation of viscous oils, high abundance of resins and asphatenes and characterized by unresolved complex mixture in chromatograms[30,123-126].

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