

# INTRODUCTION

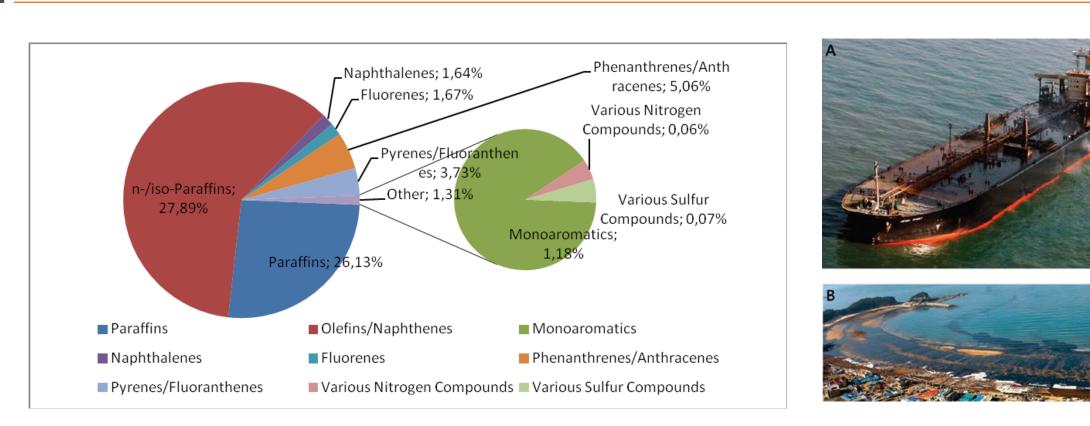
Determination of parties liable or guilty to recover costs of the cleanup and remediation, has grown dramatically in last decade, environmental forensics has emerged as a discipline directed toward this goal. Traditionally, these studies have been addressed through utilization of techniques such as gas chromatography (GC) and gas chromatography–mass spectrometry (GCMS). Biomarkers play a very important role in characterization, and source identification in environmental forensic investigations of oil spills. Biological markers or biomarkers are one of the most important hydrocarbon groups in petroleum for chemical fingerprinting. Relative to other hydrocarbon groups in oil such as alkanes and most aromatic compounds, biomarkers are more degradation-resistant in the environment. Also, biomarkers formed under different geological conditions and ages may exhibit different biomarker fingerprints. Biomarkers can be detected in low quantities in the presence of a wide variety of other types of petroleum hydrocarbons using the gas chromatography/mass spectrometry (GC/MS). Chemical analysis of environmental samples for biomarkers generates great information to environmental forensic investigations in terms of determining the source of spilled oil. This is achieved by differentiating and correlating oils, and monitoring the degradation process and weathering state of oils under a wide variety of conditions.

In recent years, stable isotopes, primarily determined using combined gas chromatography-isotope ratio mass spectrometry (GC-C-IRMS), have emerged as an equally important tool in environmental forensics. Determination of stable isotopes is not new, having been around for over 50 years. The ability to determine the isotopic composition of individual compounds in complex mixtures is relatively new and came about with the development and commercial availability of combined gas chromatograph-isotope ratio mass spectrometers (GC-C-IRMS) in the late 1980s and early 1990s combined approach of using biomarkers and isotope ratio, can lead to accuracy and confidence of the forensic conclusion of the results. As we will see in the presentation in some cases the CG-C-IRMS could be the only possible technique for identification. Depending on the samples, GC-C-IRMS can be the only reliable technique for fingerprinting due to lack of biomarkers.

## **ENVIRONMENTAL LIABILITY**

- Cause of action is bedrock of environmental litigation
- Case alleging environmental contamination must involve a thorough understanding of the science behind the allegations, including the laboratory and statistical methods employed by experts when formulating conclusions about causation or damages.
- Much like traditional fingerprint matching done by law enforcement officers to identify an individual, chemical fingerprinting relies heavily on statistics and probabilities to arrive at a measure of the likelihood that a pollutant came, or did not come, from a particular source.
- Most environmental forensic techniques can allow lawyers to definitively rule out a particular source of contamination, but they cannot affirmatively prove the source of contamination beyond all doubt.
- More recent cases have recognized stable isotope analysis as a mainstream analytical technique and accepted environmental forensic evidence based on stable isotope analysis as definitive evidence.
- The United States EPA recognizes the utility of stable isotope analysis, and says that "[s]table isotope analysis can be used in ecological studies to trace chemical movement through the environment." (https://www.epa.gov/eco-research/stable-isotope-mixingmodelspublications, last accessed, April 2, 2017).

## **CHEMISTRY OF OIL**

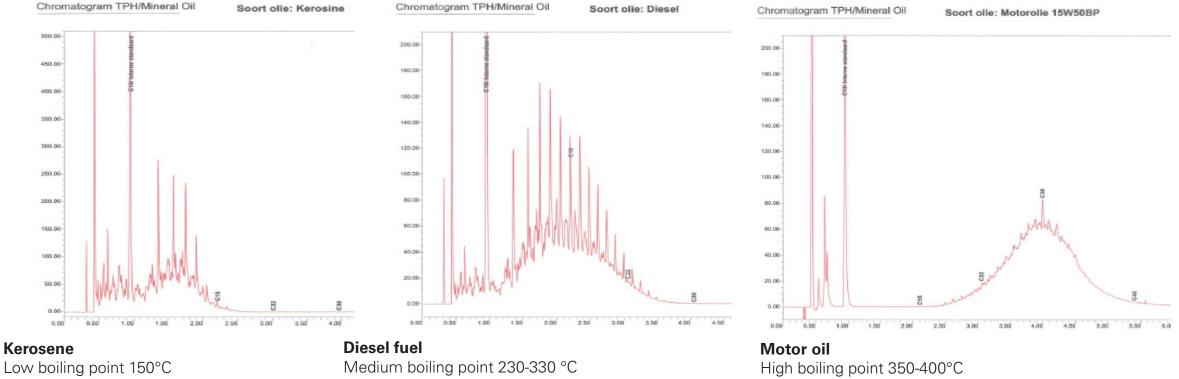


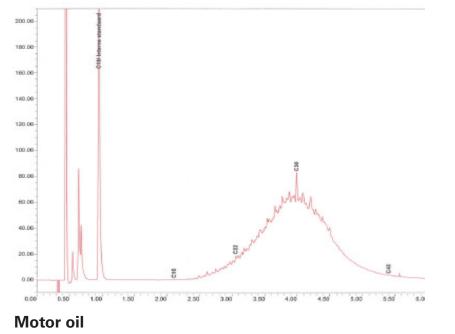
## **TIERED APPROACH TO CHARACTERIZATION**

- FINGERPRINTING AND COMPARISONS TO SOURCE OILS
- GC & GC/MS using a combination of methods
- Complex SIM GC/MS & GC/FID analysis

## Alkyl PAH's, Parent PAH's

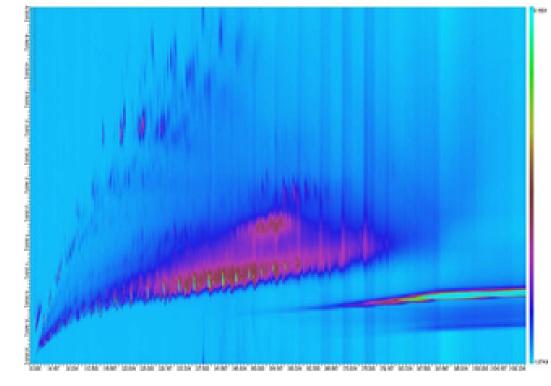
• PIANO Analysis (environmental) (Paraffins, Iso-paraffins, Aromatics, Naphthenes, Olefins Using GC or GC/MS VISUAL COMPARISON OF CHROMATOGRAMS AND CHROMATOGRAPHIC OVERLAYS

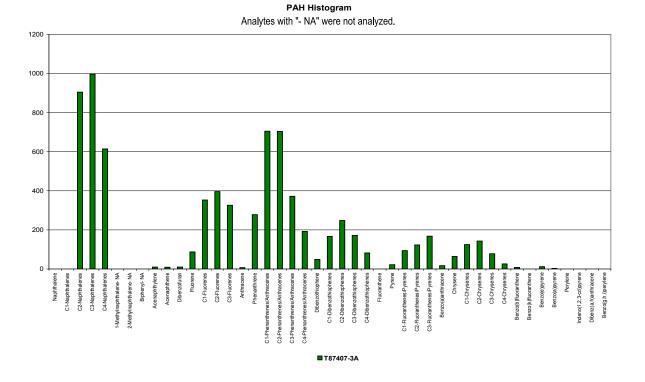


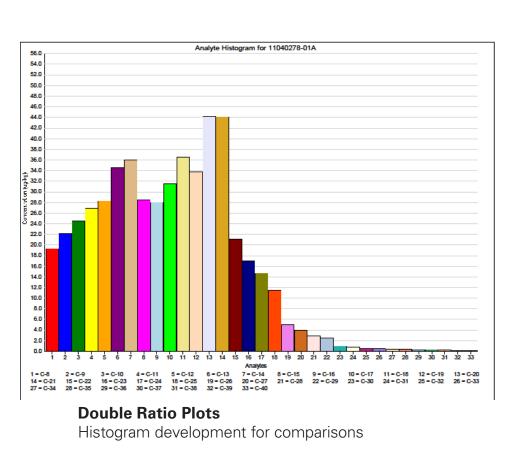




Check for characteristic compounds, Weathering checks of hydrocarbons and PAH's, Ion chromatographic comparisons Diagnostic ratio calculations for the hydrocarbons, PAH's







# **ADVANCES IN OIL SPILL FORENSICS USING BIOMARKERS AND ISOTOPE RATIO TECHNIQUE** HARRY BEHZADI, PHD SGS North America Inc.

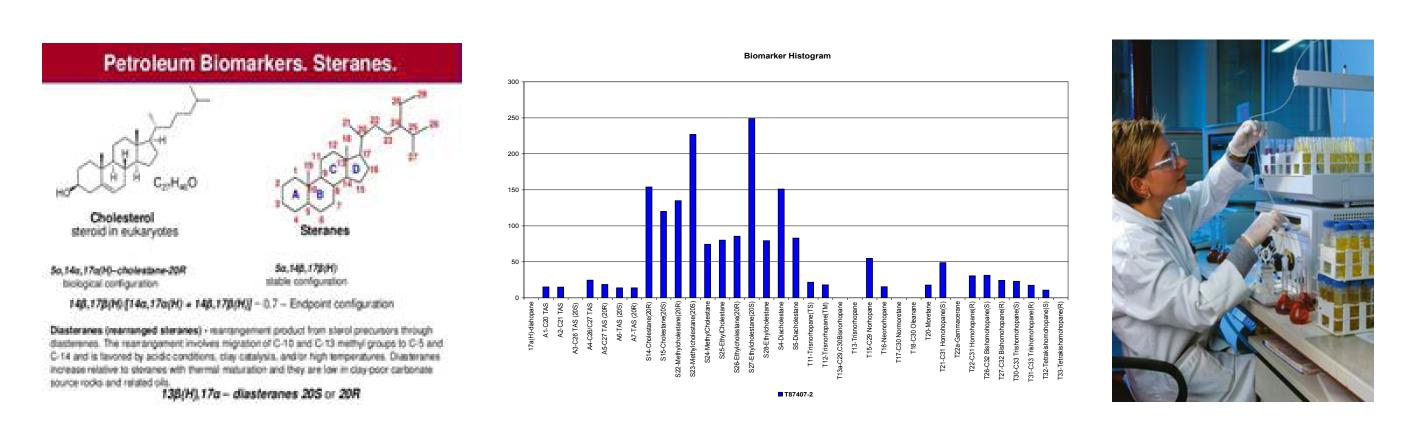
## **BIOMARKER TECHNIQU**

### WHAT ARE BIOMARKERS

Biomarkers are one of the most important hydrocarbon groups in petroleum for chemical finger printing. They are complex molecules derived from formerly living organisms. The biomarkers found in crude oils, rocks, and sediments are stable and show little or no changes in structures from their parent organic molecules, they carry information about the nature, source, type, geological conditions, and thermal history of these organisms.

Biomarkers are naturally occurring and stable hydrocarbons present in crude oils and most petroleum products (complex molecular fossils) BIOMARKER TECHNIQUE

- Oils and petroleum products from different sources may have different distribution patterns of biomarkers. Various biomarkers can occur in different carbon ranges. Also, the abundances or concentrations of individual biomarkers could be markedly different. Therefore, qualitative and quantitative comparisons of biomarker distribution are important for spill/source identification and correlation.
- o If target biomarkers detected in spill samples can be found in the same defined carbon range of
- suspected source.
- o If the distribution patterns and profiles of biomarkers are matching.
- o If the abundances of target biomarkers are matching.
- o If there are any unique or unknown biomarker.
- o If the diagnostic ratios of the major biomarkers are matching.
- In most cases, not matching among biomarker distributions is strong evidence for lack of correlation between spill sample(s) and suspected
- source(s). • However, matching may be an indication of correlation of spill sample(s) and suspected source(s), but it is not necessarily a "proof" that samples are from the same source.



## **CARBON ISOTOPE RATIO TECHNIQUE**

#### WHY ISOTOPES

Stable carbon isotopic composition (13C/12C) of oils reflects the physical and biological processes that influence oil formation and refinery, for this reason it can be used as a tool helpful in tracing the source of oil spilled in the environment.

Measurements of whole oils (EA-IRMS) and/or specific components (GC-C-IRMS) such as: n-alkanes, PAHs.

#### **ADVANTAGES**

Heavily weathered samples - isotopic compositions remain largely unaffected. Lighter refined products (absence of biomarkers such as steranes and terpanes) – may provide the ONLY reliable way of source identification for such samples

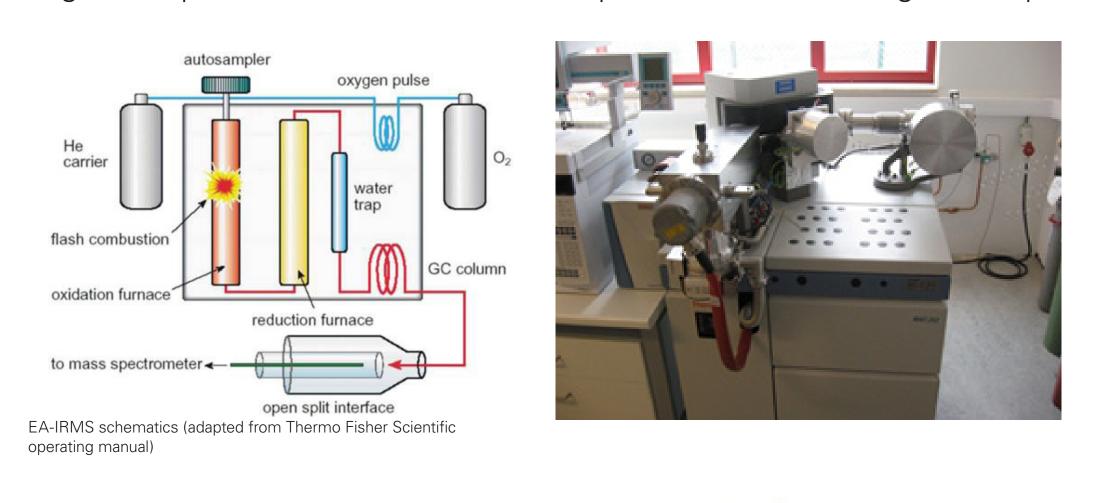
## **ISOTOPE RATIO MASS SPECTROMETRY PRINCIPLES**

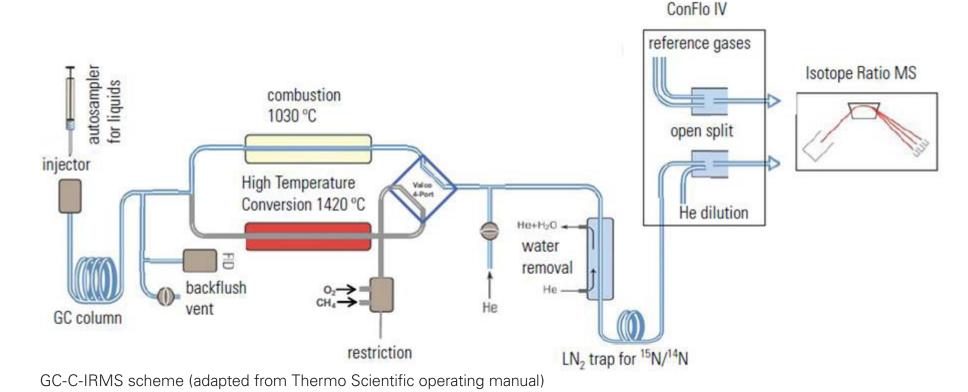
#### WHAT CAN WE MEASURE?

Isotopes are defined as atoms of the one element that differ in the number of neutrons present in their nuclei, i.e. have different mass numbers. Stable isotopes of light elements (H, C, N, O, S) at the natural abundance level

- Carbon (C) 12C Relative % Abundance 98.892, 13C Relative % Abundance 1.108
- Hydrogen (H) 1H Relative % Abundance 99.984, 2H Relative % Abundance 0.0156
- Oxygen (O) 160 Relative % Abundance 99.759, 170 Relative % Abundance 0.037, 180 Relative % Abundance 0.204

Relatively high mass difference in light isotopes causes considerable isotopic fractionation during natural processes



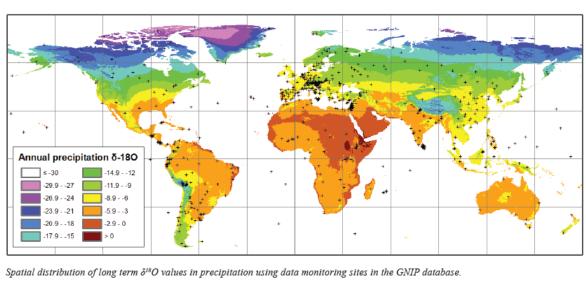


HOW?

- Online conversion of a compound(s) to corresponding gas (H to H2; C to CO2, N to N2, O to CO and S to SO2)
- Natural abundance isotope ratio data are generally quoted as delta values, δ. (e.g. 13C/12C) in a sample. This is a relative measurement
  usually made against a laboratory's reference material. Generally, δ-values are quoted relative to an internationally recognized standard that
  is arbitrarily set to 0‰. (Vienna Peedee belemnite for carbon) conventional δ notation:

$$S^{13}C(\%) = {}^{13}C/{}^{12}C_{\text{sample}} -1$$

- $^{13}C/^{12}C_{VPDB}$
- By measuring isotope 'finger prints' of samples we can (amongst others): have an insight into the geochemical processes; have an insight into the origin of a sample (environmental forensics)
- NATURAL VARIATIONS IN STABLE ISOTOPES
- Variations in isotopic ratios of water ( $\delta^2$ H and  $\delta^{18}$ O) on Earth are largely dependent on climate, thus altitude, latitude and distance from the

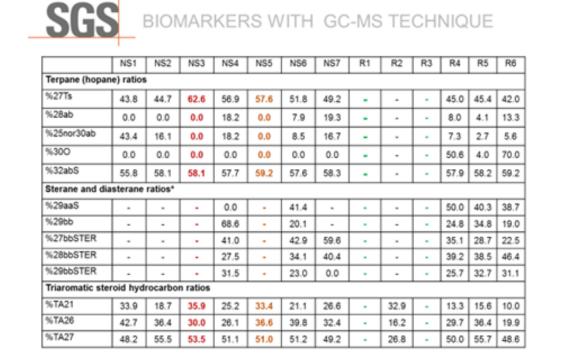


• Elemental availability depends on soil pH, humidity, porosity, clay and hemic complex, thus may as well provide markers characteristic for specific geographic origin.

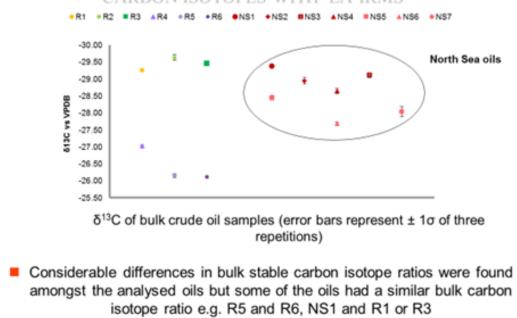
# **CASE STUDY 1**

Aim of study: to characterize various samples of crude oils, which originated from different geographical locations GEOGRAPHIC ORIGIN OF OIL SAMPLES

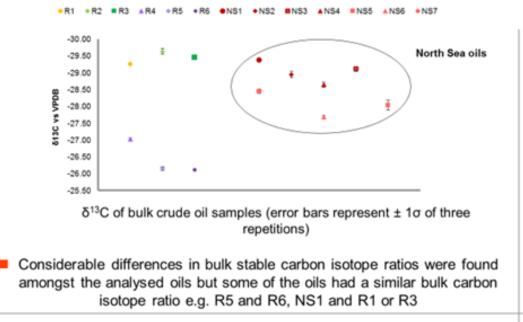
SAMPLE NAME	OIL FIELD	LOCATION	SAMPLE NAME	<b>OIL FIELD</b>	LOCATION
NS1	Clair	Northern North Sea	R1	Unknown	Algeria
NS2	Forties	Central North Sea	R2	Unknown	Unknown
NS3	Gullfaks	Northern North Sea	R3	Unknown	Libya
NS4	Brent	Northern North Sea	R4	Unknown	Bonny, Nigeria
NS5	Ekofisk	Central North Sea	R5	Burun	Turkmenistan
NS6	Unknown	Southern North Sea	R6	Оуо	Nigeria
NS7	Grane	Northern North Sea			

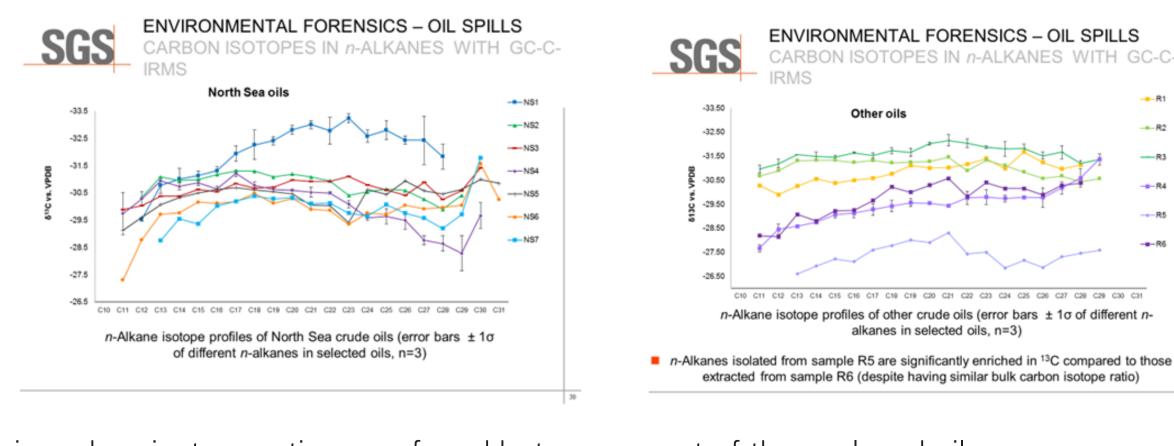


SGS ENVIRONMENTAL FORENSICS – OIL SPILLS BULK CARBON ISOTOPES WITH EA-IRMS









• Significant differences in carbon isotope ratio were found between most of the analysed oils

- Isotope n-alkane profile was helpful in discrimination between some of the oils that had a similar bulk carbon isotope ratio
- Samples R1 and R3 none of the biomarkers were found, and in R2 only one group present
- NS3 and NS5, impossible to distinguish using biomarker approach alone

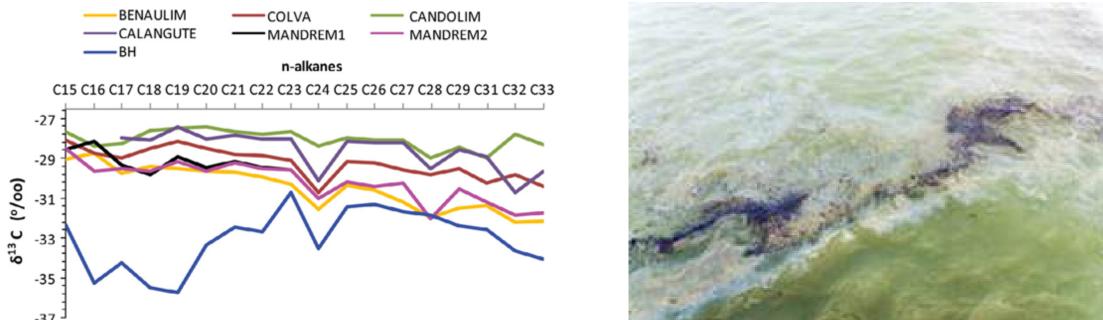


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	Contents lists available at SciVerse ScienceDirect	ARINE POLLUTION BULLETIN
	Marine Pollution Bulletin	
ELSEVIER	journal homepage: www.elsevier.com/locate/marpolbul	
	of sources of tar balls deposited along the Goa coast, Ind inting techniques	ia,
using fingerpr		ia,

Fingerprinting methods used by authors were: individual n-alkanes analysis, isoprenoid hydrocarbons (pristane and phytane), PAHs and biomarkers such as pentacyclic terpanes, additionally stable carbon analyses of n-alkanes were used

• 3 different crude oils were considered as possible sources of tar balls (South East Asian crude oil (SEACO), Middle East crude oil (MECO) and Bombay High crude oil (BH)) **RESULTS**:

Using biomarker approach, MECO could be excluded as a potential source but results were inconclusive regarding SEACO and BH crude oils





• Using stable carbon isotope analysis of n-alkanes in tar balls and BH sample, this crude oil could be excluded as well, thus leaving SEACO as a most probable source

## CASE STUDY 3

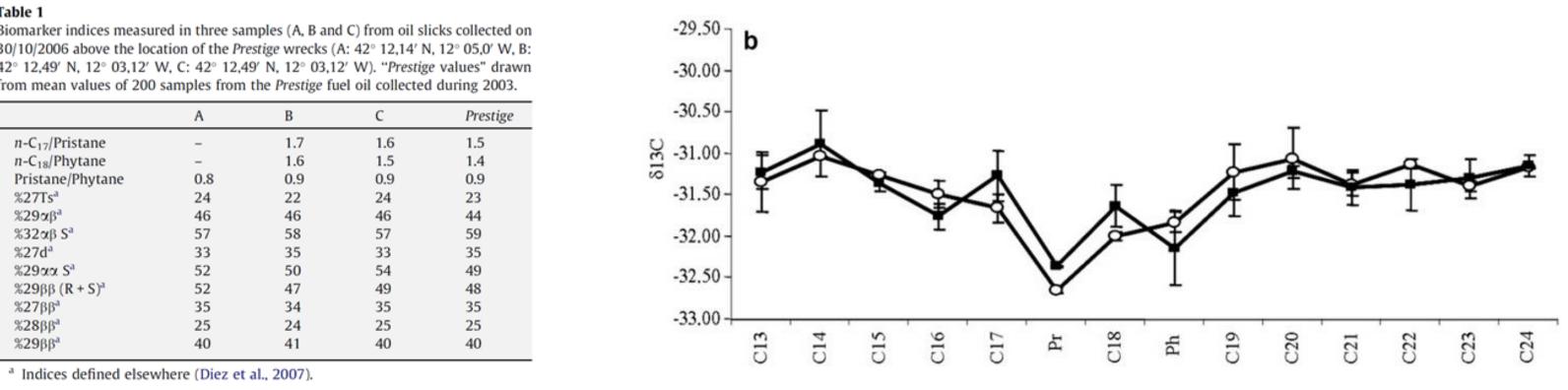


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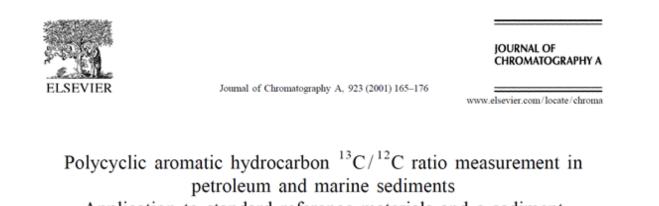
• Authors sampled oil slicks and tar balls from water above the Prestige oil tanker ship wreck 4 years after the accident

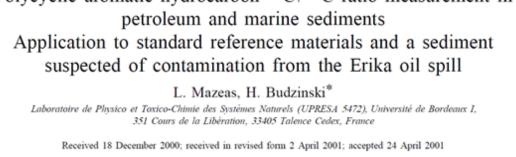
• Fingerprinting techniques used were: n-alkane and biomarker indices analysis coupled with stable carbon isotopes analysis of n-alkanes RESULTS

Using n-akanes and biomarker approach as well as stable isotope analysis, the oil slicks were identified as deep-sea leaks from the ship wreck despite the sealing of the wreck cracks performed in 2003



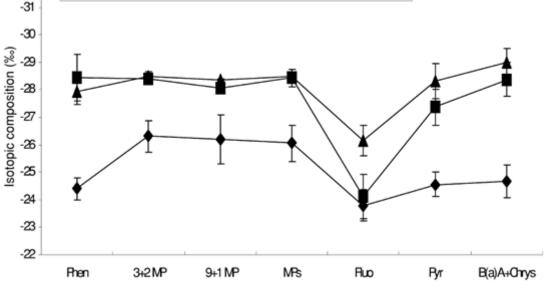
## CASE STUDY 4





• Authors used stable carbon isotope measurements of polycyclic aromatic hydrocarbons (PAHs) in sediments suspected to be contaminated with oil spilled by Erika tanker in 1999 (French coast) RESULTS

Comparison of isotope profile of PAHs from contaminated sediment with the product carried by the tanker provides a clear proof that, indeed, the sediment was affected by the Erica spill





## CONCLUSION:

Carbon isotopic ratio can provide useful information about oil spill and oilfield fingerprinting

Combined approach with biomarker methodology (EN 15522) leads to increased confidence in the results

Depending on the samples, GC-c-IRMS can be the only reliable technique for fingerprinting due to lack of biomarkers. **APPLICATION:** 

In cases of pollution whose source is unknown but a limited number of suspects are known, the combination of before mentioned techniques can provide analytical data for source recognition.