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Analysis of water soluble fractions of crude oil by gas chromatography: Mass spectroscopy

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Abstract
Crude oil is the major source of energy in the modern society to meet the global energy demand by which exploitation of crude oil and its transportation increasing rapidly leading to frequent catastrophic oil spills. When oil spill occurs or when oil is discharged into aquatic environment the components of the crude oil present in it are mostly volatile, evaporates into the environment and the fraction of the oil soluble in water (i.e., WSF) is available to the organisms directly which is the main determinant of crude oil toxicity to aquatic organisms. Although this fraction is present only in relatively low concentrations, it is this fraction which is in most intimate contact with fish and other pelagic organisms with carcinogenic and mutagenic potential. So, the aim of the study was to determine different hydrocarbons dissolved in water soluble fraction (WSF) of crude oil qualitatively and quantitatively. The determination of different hydrocarbons present in water soluble fraction of crude oil can be used as reference point for many studies in future for determining the toxicity of water soluble fraction of crude oil to fishes.

Keywords: Water soluble fraction, crude oil, gas chromatography

Introduction
Marine ecosystems are the largest and highly productive ecosystems on earth and thus pollution in marine environment is receiving worldwide attention. Since the last decades many maritime accidents occurred in sea resulting in major contamination of environment with crude oil components disturbing the entire ecosystem for long period of time. (Torrey canyon, 1967; Amoco Cadiz, 1978; Atlantic Empress, 1979; Deepwater Horizon, 2010; Bohai Bay, 2011; Ennore oil spill, 2017; Sanchi oil tanker collision, 2018; MV Solomon Trader fuel oil spill, 2019). There are many techniques ongoing to limit the impact of such oil spills like use of dispersants, skimmers, sorbents, bioremediation etc., to recover fastly from oil spills and to protect marine life, but once when oil is spilled over the sea, the fraction of oil soluble in water is immediately absorbed by aquatic organisms which is the main concern for crude oil pollution. Even though this fraction is present in low concentrations it is this fraction which is directly available to aquatic organisms which is the main determinant of crude oil pollution. According to the literature oil spills have mostly large-scale consequences such as the loss of biodiversity and ecosystem services (Sanchez *et al.*, 2006; Antonio *et al.*, 2011) [11, 2]. In water, petroleum undergoes physical and chemical processes (e.g., evaporation, dissolution, emulsion, photolysis, biodegradation) which generate a water-soluble fraction (WSF) (Daling *et al.*, 1990; Perez-Cadahia *et al.*, 2004) [4, 8]. The WSF of petroleum and its derivative products contain a mixture of polycyclic aromatic hydrocarbons (PAH); monoaromatic hydrocarbons often referred to as BTEX (benzene, toluene, ethylbenzene and xylenes); phenols; heterocyclic compounds containing nitrogen and sulfur (Saeed and AI-Mutairi, 1999) [12] and heavy metals (Rodrigues *et al.*, 2010) [10]. Although the more toxic compounds are volatile, fish can quickly absorb part of the WSF with adverse consequences to biological organization (Collier *et al.*, 1996) [3]. In aquatic organisms, WSF of petroleum may impair physiological integrity and its chemical compounds may be disseminated throughout the food chain. Another important toxicological aspect of WSF is its high polyaromatic hydrocarbon concentration, which has carcinogenic potential (Khan *et al.*, 1995) [6]. Investigation of the toxicity of crude oil contamination has revealed that the toxicity 10 of oils is apparently due to the water-soluble fraction (WSF) rather than to dispersed droplets (Rice *et al*., 1977) [9]. So, it is important to determine type of hydrocarbon and concentration of particular hydrocarbon present in water soluble fraction of crude oil to determine the toxicity of water soluble fraction of crude oil and its effects on aquatic organisms.

Materials and Methods

Toxicant used

The crude oil used in the study for the estimation of hydrocarbons dissolved in water soluble fraction (WSF) is Iranian heavy crude oil which is obtained from Mangalore Refinery Petrochemicals Limited (MRPL); Mangalore India. The industrial information of crude oil is as follows: specific gravity- 0.8783, API calculated- 29.6 and viscosity@ 50 ◦C- 9.104.

Preparation of water soluble fraction of crude oil

Two Water soluble fractions (WSF) of crude oil were prepared in the ratio of 1:9 and 1:19 in which 1 part of crude oil was mixed with 9 parts of deionised water which represents 1:9 ratio and 1 part of crude oil was mixed with 19 parts of deionised water for 1: 19 ratio with the help of filters in 50 litre aquarium tanks (figure.1). The mixtures were completely kept in motion with proper mixing of oil and water for 20 hours and the aquarium was capped with polythene sheet to minimize the evaporation of hydrocarbons. Then the mixture was left undisturbed for 4 hours, the top oil layer was discarded and the water in the aquarium was collected which is technically called as water accommodated fraction (WAF) of crude oil. Then the water was filtered through

0.45 µm pore-size membrane filter which absorbs the residuous oil in the water and the solution now is technically called as water soluble fraction (WSF) of crude oil. The prepared Water soluble fraction stock was used for the analysis of dissolved hydrocarbons by Gas chromatography mass spectrometry (GC-MS) for the present study. Preliminary data indicated that 10 ml oil per litre of water (1:100) was sufficient to produce a water soluble fraction saturated with most compounds other than benzene and toluene. For this investigation it was decided to use a oil to water ratio of 1:9 and 1:19 fractions.

Analysis of Total hydrocarbons in Water Soluble Fraction

The concentration of total hydrocarbons in water soluble fraction (WSF) of crude oil samples were analysed qualitatively and quantitatively at Centre for Cellular and Molecular Platforms; Bangalore, India by using GC-MS. The method used for sample preparation of water soluble fraction of crude oil for analysis by gas chromatography is liquid-liquid extraction.

Instrumentation-GC-MS set up:

Instrument	Perkin-EImer clarus 680 SQ8 with autosampler
Column	RTX-5MS 30M, 0.32mm ID
Mobile phase A	Helium
Flow rate	1ml/min
Injection volume	1µl
Split	Splitless
Run time	23min
Configuration	Autosampler-GC-MS

Temperature Program

Conditions	◦C/min	◦C	Hold time
Initial	--	50	2
Ramp1	8	150	0
Ramp2	20	280	2

Protocol for sample preparations by liquid-liquid extraction:

For the analysis of total hydrocarbons present in water soluble fraction of crude oil, 100ml of water soluble fraction was taken and to it known amount of internal standard., 172µg of 1- phenyloctane was added. To the prepared solution 1ml hexane was added and magnetic bead was immersed in the flask for continuous stirring at 300rpm for 24hrs at room temperature, after stoppering the flask and sealing it with teflon. Hexane layer was recovered and concentrated to 100µl and 1µl of concentrated extract was analysed by GC-MS in splitless mode. Hydrocarbon concentrations were estimated relative to the amount of internal standard added and the response (peak area) measured.

Results and Discussions

Qualitative and quantitative estimation of WSF of crude oil was done by liquid-liquid extraction and the results of the analysis are tabulated in table 1. Concentration of hydrocarbons dissolved in 1: 9 fraction is greater than 1: 19 fraction of WSF of crude oil as oil to water ratio is high in 1: 9 concentration. Gas chromatograms showing different compounds with different peaks were indicated in figure 2 and figure 3. Different hydrocarbons were determined according to retention time of the particular hydrocarbon with more of aromatic hydrocarbons dissolved in WSF of crude oil.

The majority of investigators have measured the toxicity of their WSFs by analysis using UV, IR or fluorescence spectroscopy, and have indicated that the WSF was toxic to the various species tested. These observed toxicities would not appear to be associated with the volatile fraction, since no precautions were taken to preserve this extremely volatile material, but rather with the higher boiling point components usually measured by the microextraction method. The most concerning toxicity of Polycyclic aromatic hydrocarbons (PAHs) is their carcinogenicity. Low-molecular weight compounds (naphthalene) are dominantly found in the water soluble fractions of crude oil due to their relatively high water solubility. The results of the present study are in agreement with Andres J. Rascon *et al.*, 2019 [1] who also determined presence of naphthalene in fish meat using GC-MS. The most volatile and water-soluble aromatic hydrocarbons which includes benzene, toluene, ethylbenzene and xylenes (BTEX) are well detected in both the fractions of water soluble fractions of crude oil. Many toxicological studies have revealed various types of adverse effects associated with the water soluble fraction of PAHs to fishes: developmental toxicity, genotoxicity, immunotoxicity, oxidative stress, and endocrine disruption (Masota Honda and Nobuo Suzuki, 2020) [7]. Examination of the data from the tables and comparison of the chromatograms of the different concentration of crude oils gave the clear impression that the WSFs were all very similar, with the only differences being the relative abundance of some compounds.

Table 1: Qualitative and Quantitative data of hydrocarbons dissolved in 1:9 and 1:19 ratio of water soluble fractions of crude oil

Retention time (RT)	Compound	Concentration in 1:9 fraction (µg/100ml sample)	Concentration in 1:19 fraction (µg/100ml sample)
4.5	Toulene	5.41	-
6.68	o-xylene	28.92	20.05
6.9	p-xylene	19.13	4.17
7.18	p-xylene (2)	7.13	4.25
8.81	1-ethyl,4-methylbenzene	9.20	4.63
9.21	1-ethyl,2-methylbenzene	11.99	5.46
9.6	Mesitylene	15.10	10.07
10.3	1,3,4-trimethylbenzene	9.36	6.03
10.57	Indane	1.91	--
11.17	1,3-diethylbenzene	3.41	1.68
11.46	1-methyl,3-propylbenzene	2.25	0.99
11.76	1-ethyl,3,5-dimethylbenzene	3.62	1.28
11.97	p-cymene	2.62	1.53
12.35	undecane	3.25	1.40
13.71	1,2,3/4,5-tetramethylbenzene	6.07	4.04
14.53	Naphthalene	5.87	4.71
14.6	L-alpha-terpineol	0.42	--
14.83	2,4,6-trimethylphenol	3.22	1.17
16.39	1-methylnaphthalene	4.58	3.09
16.61	2-methylnapthalene	5.48	4.07
17.86	1,7-dimethylnaphthalene	1.83	1.79
17.92	1,3-dimethylnaphthalene	3.38	3.01
18.09	1,4-dimethylnaphthalene	2.02	1.34

Fig 1: Preparation of water soluble fraction of crude oil

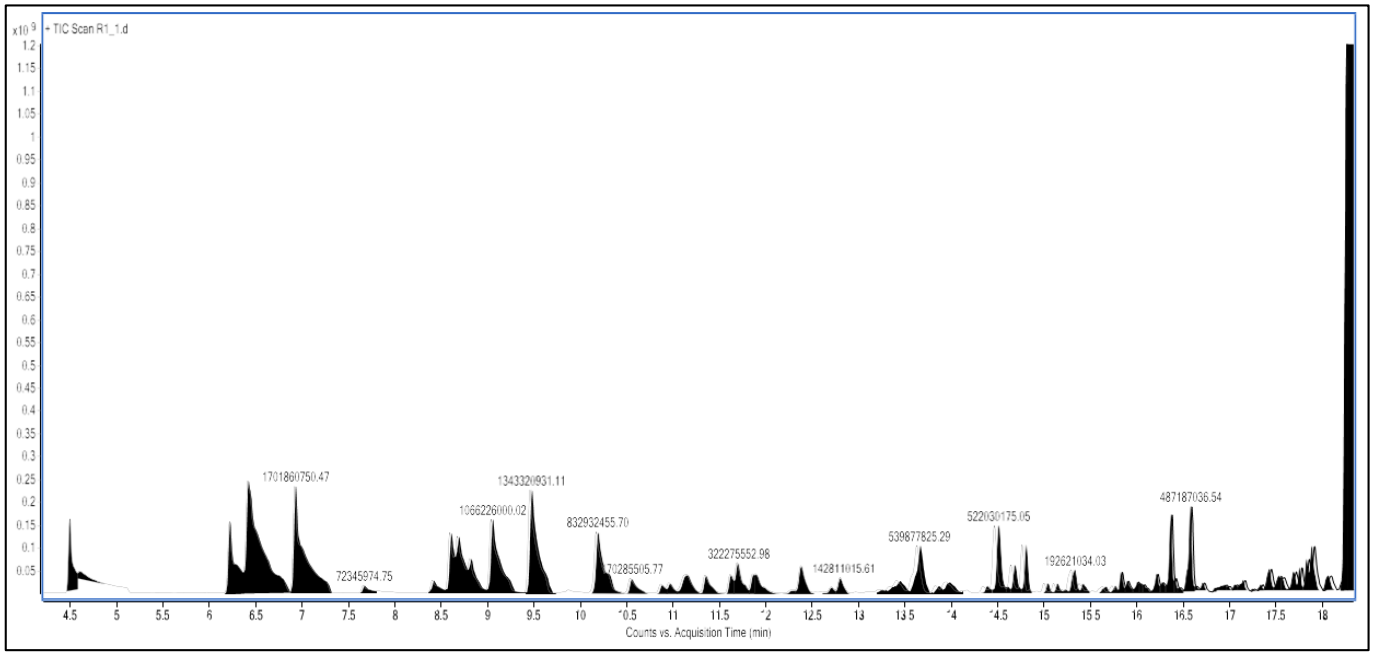

Fig 2: Gas chromatogram of 1:9 ratio of WSF of crude oil

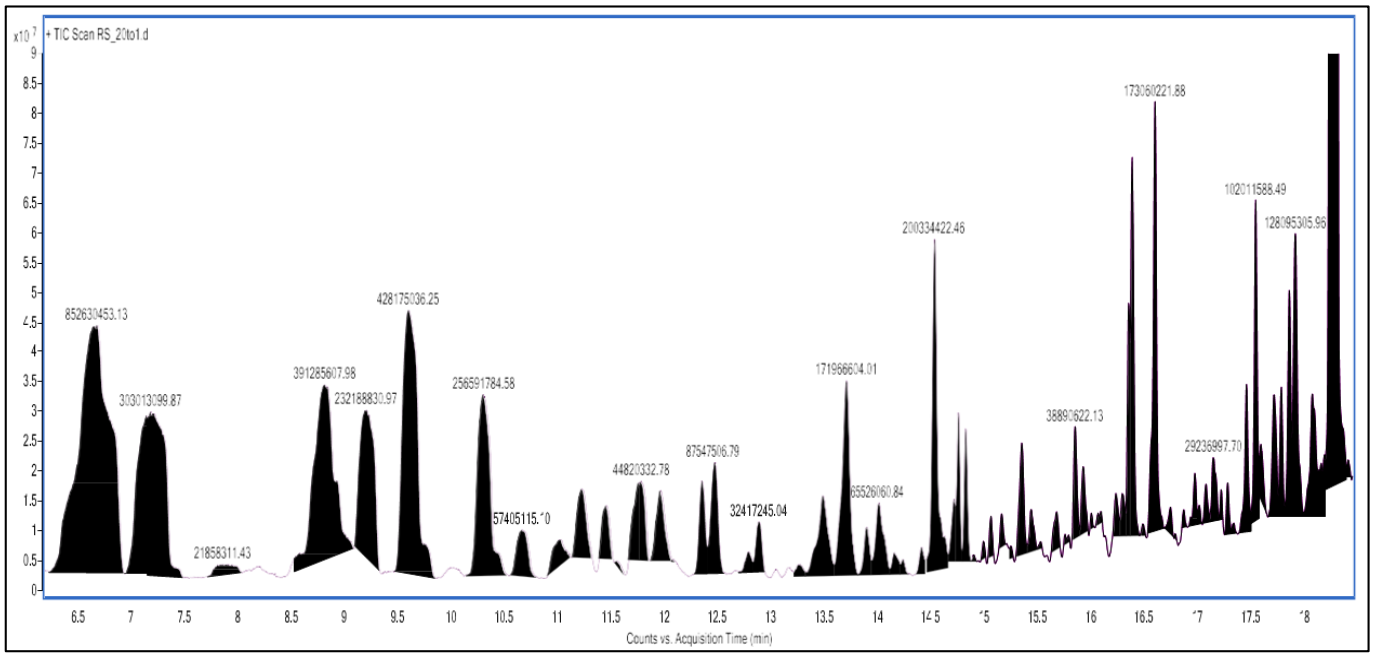

Fig 3: Gas chromatogram of 1:19 ratio of WSF of crude oil

Conclusion

No single analytical technique could measure all the components of dissolved organic material in a WSF prepared from a crude oil or petroleum product as the compounds are mostly volatile. Headspace analysis was particularly suitable for measuring the extremely volatile fraction, while a solvent extraction was preferable for the higher boiling point components. Aromatic hydrocarbon concentrations are more in the both the ratios of crude oil with higher concentration in 1: 9 ratio. Several researchers found that PAHs present in crude oil are responsible for toxic effects such as immunotoxicity, embryonic abnormalities and cardiotoxicity for wildlife including fish, benthic organisms, and marine vertebrates. So, the analysis of hydrocarbons present in WSF of crude oil in the present study can be used as reference data for determining individual toxicities of hydrocarbons present in WSF of crude oil for monitoring of crude oil pollution in aquatic water bodies and their effects on aquatic biota.

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