DETERMINING THE LETHAL AND SUB-LETHAL AQUATIC TOXICITY OF SUNLIT JET OIL USING COPEPOD AS TEST ORGANISM, AND EVALUATION OF PDMS AS AN ALTERNATIVE TO ANIMAL TESTING.

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A Thesis Submitted to the IESE in partial fulfillment of the requirements
for the degree of
MASTERS OF SCIENCE
IN
ENVIRONMENTAL SCIENCE

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Dedication

“I dedicated this thesis to my beloved parents, who taught me moral and ethical values, encourage me and trusted in my abilities and stood beside me at every moment in my life”

ACKNOWLEDGEMENTS

Thanks to ***Allah Almighty*** “Most Beneficent” Who blessed us with ability, skills and wisdom to accomplish this research.

I express my sincere gratitude to my parents for their affection, prayers, moral and financial support throughout my academic career which enabled me to chase the butterflies of their sweet dreams.

My Utmost gratitude to my supervisor ***Dr. Deedar Nabi*** for his supervision, guidance, patience and continuous support. Special thanks to my guidance committee members, ***Dr. Imran Hashmi*** and ***Dr. Muhammad Arshad*** in particular for their assessment and adding constructive comments throughout my research.

I would also like to acknowledge my Husband ***Arif Hussain Shah*** for being always with me. Special gratitude to my friends Misbah khan, Sakina Fatima, Fakhra Muneeb, Shanza, Ayesha Ashfaq, Maria Zafar, Nisar Ali Khan, Usman Maqsood, Irfan Khawar, and Muhammad Irfan for their immense support throughout the course of this research. Sincere thanks to the lab staff of Environmental Chemistry and Biotechnology lab, IESE NUST.

Abstract

Photo-oxidation is one of the important environmental weathering processes that changes the chemical composition of spilled oil. This study examines the effect of sunlight shined on the spilled oil, and reports toxicity of transformation products of native compounds in the oil mixture. The toxicity due to transformation products was experimentally determined using copepods as test organisms, and this was compared to the native hydrocarbons parent compounds. Photo generated transformation products of light jet oil was also determined. Our experiments show that the photo generated transformation products are more toxic to copepods at lethal and sub lethal endpoints. However, this change in the toxicity depends on exactly where the oxygen atom is added in the carbon backbone of the parent compound. This study highlights that the photo generated transformation products are environmentally relevant family of chemicals, which should not be ignored during risk assessment of oil spills in the environment. For millions of years animals are used as test objects for risk assessment aid, to compile with the regulatory authorities like REACH and US EPA. Using animals for toxicity assays is an ethical implication. In this study a new model is developed to see PDMS can be used as an alternative to animal testing and how much passive sampling phases resembles toxicity end points. For hydrocarbons, the model shows root mean square error (rmse) of

0.30 log unit when compared to the experimental values (n=46) reported in literature. Whereas previous model of critical membrane concentration returned higher rmse value (0.35 log unit) for same experimental dataset (n=46). Model for oxygenated hydrocarbons (OxHCs) transformation products which were formed in weathered oil samples due to photo oxidation, consisting of ketones, aldehydes, alcohols and acids, show higher rmse values indicating these chemicals have higher toxic mode of action than narcosis.

Key words: Photo oxidation, Transformation products, Hydrocarbons, Oxygenated hydrocarbons, jet oil, Bioassays, Polydimethylsiloxane

Contents

List of the Abbreviations

EPA	Environmental Protection Agency
GC- MS	Gas chromatography–mass spectrometry
DWP	Deep water Horizon
GoM	Gulf of Mexico
NRDA	Natural Resource Damage Assessment
TPH	Total Petroleum Hydrocarbons
ITOPF	International Tanker Owner Pollution Federation
BCF	Bio concentration factor
EU	European Union
BTEX	Benzene, Toluene, Ethyl benzene and Xylene
REACH	Regulation, Evaluation and Authorization of Chemicals
WAF	Water Accommodated Fraction
NOAA	National Oceanic and Atmospheric Administration
PDMS	Polydimethylsiloxane
CROSERE	Chemical Response to Oil Spills Environmental Research Forum

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Chapter 1

Introduction

1. 1 Global Issue of Oil Spill

Release of crude oil and its products into the environment has resulted in many problems that are of global concern. The rise in world energy demand during the last decades and the growing use of petroleum products have resulted in enormous exploration, production and transportation of petroleum in the sea, making marine environments especially susceptible to increased risk of crude oil spills (National Research Council, NRC 2003; Dalsøren et al. 2007). Input of organic load continuously in coastal ecosystems provide compounds that are potentially available for uptake and bioaccumulation by marine organisms and are the main source of chronic toxicity (Roberta L). Composition of oil depends on the area in which it is obtained, however, it is composed of tens of thousands of compounds including alkanes, alkenes, alkynes and aromatics in different proportions (R. James et al), in a complex manner whose chemical composition is being changed because of different weathering processes (Hyun-Joong Kang). On the surface of water in oceans photochemical degradation of crude oil clean the organic contaminations and is more important than biodegradation and microbial degradation, the latter processes themselves being promoted by photo degradation (Yang Guipeng). Bioassays reveal that residuals of oil contain many important fractions of mono- and polycyclic aromatic hydrocarbons which play an important role in terms of eco- toxicity of oil spill (Grote, M, Jiang, Z).

Crude-oil spillage has a serious hazard to human being and the ecosystem. The associated adverse impact includes intensification of the greenhouse effect, acid rain, water quality impairment and groundwater contamination which becomes unfit for long time use. It contributes to air pollution through gas flaring resulting in respiratory diseases (Jike, 2004). It also poses risks to human health, biological environment and vegetation. Skin rashes, headaches, eye and throat irritation, and some of the commonly reported symptoms of oil spill adverse health effects include malaise. Birds and mammals are also vulnerable to oil spills when their habitats become contaminated thereby reducing their reproductive rates and survival as well as triggering physiological impairment (EPA 2008). Various eco-toxicological impacts of oil spills on the ecosystem have been intensively studied to assess baseline toxicity (Di Toro).

Among the planktonic components of marine ecosystem, copepods are of most abundant and dominant community (Longhurst 1985; Humes 1994). Many species of fish and fish larvae are main prey of them (Last 1980). In the transfer of matter from low to higher trophic levels in marine food webs planktonic copepods play a crucial role (Banse 1995). Study on the effect of dispersed and weathered oil on planktonic organisms is limited. We examined the potential for the copepods to weathered jet oil of different time exposure of 5, 10, 15, 20, 25 and 30 minutes. The aim of our study is to evaluate the lethal and sub lethal health impacts of small fraction petroleum jet oil on zooplanktons. The current study was carried out in controlled laboratory conditions so that direct observation of photochemical transformations of jet oil composition could be observed on copepods. This study evaluated the lethal and sub lethal effects of a large oil spill on zooplanktons. Increasing toxicity with increased weathering of oil was clear from result.

1. 2 Area of Study

Area of study in this research is the Misriot dam, a beautiful mini dam with a village, situated in the South of Rawalpindi. It takes half hour to reach Misriot dam through Dhamial Road that is joined by Chakri interchange.

Misriot Dam is built in 1963 during the reign of President Ayub Khan to water the surrounding areas of Potohar Plateau. The spill ways of Misriot dam provide water to the surrounding agricultural fields. It has abundant fishes and rich productivity of zooplanktons because of input of huge organic matter from the surrounding agricultural

Fields. Abundance in fish population and zooplanktons fluctuates in different seasons. In this study area, maximum density of copepods was recorded in spring and summer.

1. 3 Test Animal

1.3.1 Copepods

Copepods are simply small group of crustaceans found in sea and fresh water. They are also found in salty habitat. Their body is only 0.5-2 mm (0.02- 0.08inches) in length, are colorless, and transparent so difficult to see with the naked eye. Some copepods are planktons, other are benthic and limno terrestrial. Planktonic copepods are very important for the marine water bodies because they are major source of food for the small fish either directly or indirectly. Copepods consume microorganisms and algae (Kraul, et al 1993) (Santhanam, Pet et, al). Sudden shifts in temperature and salinity, affecting the population of copepods as they are adopted to changing environment Copepods have the ability to adapt these sudden changes in marine environment which make suitable temperature for rearing their larvae (McKinnon et al., 2003). Life stages of copepods include four main stages, egg, nauplius, copepodite and adult. Nauplier and copepodite morphs to multiple stages to transform into mature copepod capable of interacting with marine environment (Michael Milione).

1.3.2 Selection of Test Animal for this Research

The test organisms of this research are Diacyclop Bicuspidatus odessanus, a species of cyclop copepod indigenous to Misiroit Dam, Rawalpindi, Pakistan. Its distinguishing characteristics including Antennules consist of 14 segments. These organisms have five legs with two segments and distal segment has an apical setae and an inner sub apical spine, having long caudal rami. The inner margins do not have hair.

This species was chosen to check its lethality and sub lethality (swimming pattern and respiration rate) due to its existing board information about its ecological role in marine ecosystem. One is slow, steady movement that is accomplished using their mouthparts and another is like a succession of jumps separated by stillness carried out by the appendages on the thorax. (Koehl and Stickler, 1981)

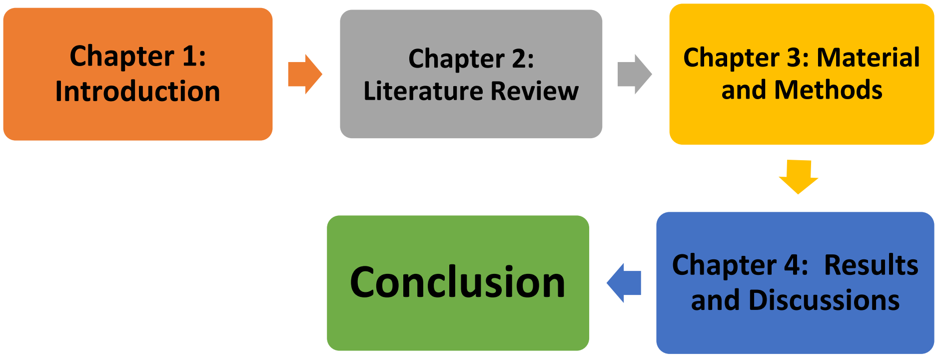
1.3.3 Objectives:

The main objectives of this research are highlighted below.

- To track changes in the chemical composition and photo generated transformation products of light jet oil using GC/MS.
- To assess reactive toxicities, if the sunlight enhances or reduces the overall toxicity of crude oil after spill.
- To conduct partitioning-based passive sampling phases akin to bio membrane for the uptake of petroleum chemicals? Answer in a new model for the estimation of toxicity of petroleum compounds.

1. 4 Organization of Thesis:

The complete research is organized in five different chapters as mentioned below, and finally the recommendations and conclusion.



Chapter 2

Literature Review

2. 1 Worldwide Oil Spills History

Countries having Pollution due to oil of water bodies is a critical problem in enormous areas of world. Exploration and production of oil along with its shipping all over the world increased the risk of oil spills. It is a great challenge to the aquatic ecosystem. Oil spills cause enormous destructions, disruptions and damages to environment, and lethal and sub lethal health effects to humans and animals (biota and abiotia). The first oil spill in the history was Torrey canyon in 1967 on the Isles of Scilly of Cornwall. It was a big tanker oil spill. The British military bombed the wreck to burn the oil. 10,000 tons of dispersants were sprayed to oil which damaged the aquatic life. (Simpson 1968) (Arne Jernelov 2000). In the recent past the marine environment has been subject to large-scale intentional releases of oil. The largest intentional oil spill in the history was during the Gulf war in 1991, in Kuwait. Iraqis planned to roast their enemies in flames of oil, for which they emptied all the oil-storage tanks along the coast, about 1200000 of oil was intentionally discharged into Gulf. War took a different turn when enemies did not come that way, leaving the Persian Gulf with disastrous contamination (linden and Jernelov 1999) (Arne Jernelov 2000). In the Gulf of Mexico, the first oil spill event occurred in 1979 on the Bay of Campeche, Mexico. The oil well was finally capped 290 days after the blowout (Shaojie sun). However, the adverse effects of Ixtoc oil spill and its long term effects on aquatic habitat were not studied thoroughly, until now information about it is unavailable (Shaojie Sun). After 31 years of Ixtoc oil spill another accident, the Deep -water Horizon, occurred in the Gulf of Mexico. During the Deep-water Horizon (DWH) spill of 20th April to 15th July 2010 five million barrels of oil andgas discharged into the Gulf of Mexico (GOM). It lasted for 87 days and caused a large sub surface release. Natural Resource Damage

Assessment (NRDA) collected samples for analyses for TPH (total petroleum hydrocarbons) and PAHs, BTEX and reported results Boehm et al. (2011, 2012). Soon after oil explosions, large volumes of oil appeared at the surface, but larger volume of oil may be suspected to be trapped in deep water. It is very difficult to categorize and identify change in the chemical composition of this portion due to physical and microbial processes. Some largest marine oil spill include Eco flask oil spill Norway in 1977, Funia 5 Nigeria 1980, Nowruz Iraq in 1983, oil tanker Exxon Valdez struck in 1989 in Alaska, Hebei Spirit oil spill 2007 in Taean County, South Korea and Montara Australia in 2009. Oil seepages occur at thousands of places and using remote sensing it has been estimated that 70,000 tons of natural oil seepage occurs in Gulf of Mexico while 20,000 in California and Alaska, (Keith et, al 2002). Whereas, in South African and Middle Eastern countries seepage of oil, spill of oil during drilling mud, and other operational activities happens on and off.

Table 2. 1: List of some largest oil spills in world (International Tanker Owner pollution Federation (ITOPF)

Position	Country	Name	Location	Year	Toned spilled (M gal)
1	Iraq	Gulf war (Kwuait)	Ist Gulf war	1991	500
2	Mexico	BP's *Deepwater Horizon* Oil Spill	Gulf of Mexico	2010	206
3	South of Texas	The *Ixtoc 1* oil spill	Pemex Ixtoc 1 platform	1979	140
4	Trinidad and Tobago	Gulf of Paria (coast of Tobago)	Atlantic Empress Greek Tanker collision	1979	88
5	Persian Gulf	The Persian Gulf War Oil Spill	Tanker platform collision	1983	80
6	France	The *Amoco Cadiz* Oil Spill	English Channel	1979	70
7	South Africa	*The Castillo de Bellver Oil Spill*	Cape town	1983	80

2. 2 Status of Oil Spills in Pakistan

In the south, Pakistan has a God gifted 1,200 kilometers lengthy coastline with the Arabian Sea which joins oil line of Persian Gulf with Indian Ocean. Pakistan have three major seaports along Arabian Sea that include Qasim port, Gwadar port and Karachi port. Gwadar port is the deepest seaport in Pakistan. Sea ports of Pakistan are used for Trade between different countries including trade of oil. Pakistan have limited resources of oil and gas; the country has heavy dependence on imported oil. Pakistan also import oil from Gulf countries through Arabian Sea especially from Saudi Arabia, UAE, Qatar, Kuwait and Iran. (khanji hajijan)

In the history of Pakistan many oil spills incidents occurred which caused disastrous environmental effects. The biggest oil spill event in history of Pakistan is Tasman oil spill. The Greek tanker on 23 July 2003, "Tasman Spirit," was carrying 67,000 tons of Iranian light crude oil from Iran to Pakistan. On 13 and 14 August 2003 the tanker spilt and released a total of 35,000 tons of crude oil into sea. (Naveed Z. Janjua)

Recently on October 2018 an oil spill on coast specifically from Mubarak Village to Churna Island was reported which is still causing major problems for the marine life and residents of the area. It was claimed the oil spill was due to burst of underwater pipelines of Byco's but they denied the claim and said their pipes are not damaged (Ramez Ahmed 29 Oct, 2018). Similarly, an oil spill on land accident occurred on 25 June 2017 in Bahawalpur where an oil tanker exploded and burned its 40,000 liters cargo, killing 219 people.

2. 3 Composition of Crude Oil

Oil either crude or fuel oils consist of millions of organic compounds including Poly Aromatic Hydrocarbons (PAHs) that have different chemical and physical properties like their lipophilic and hydrophobic properties. Crude oil is mixture of all liquid, gaseous, and solid (e.g. paraffin) hydrocarbons. Under surface pressure and temperature conditions, lighter hydrocarbons such as methane, ethane, propane and butane occur as gases, while heavier ones above butane are in the form of liquids or solids. Proportion of light hydrocarbons in crude oil varies from 97% in lighter oil to 50% in heavier ones. The hydrocarbons in crude oil include alkanes, cyclo alkanes while the other organic compounds contain nitrogen, oxygen and sulfur, and trace amounts of metals such as iron, nickel, copper and vanadium. Four different types of hydrocarbon molecules appear in crude oil. The relative percentage of each varies from oil to oil, determining the properties of oil.

Table 2. 2: Composition of Crude oil

	Composition by weight	
Hydrocarbon	**Average**	**Range**
Paraffin's	30%	15 to 60%
Naphthenes	49%	30 to 60%
Aromatics	15%	3 to 30%
Asphaltics	6%	remainder

Beside oil spills, incomplete combustion of coal, oil, petrol and wood are also responsible for the release of PAHs. Tiny soot particles act as carrier for the long distance transport of PAHs in atmosphere whereas rainout or fallout processes are used to deposit them. However, water solubility of most PAHs is considerably low (Sanders et al., 1993). (Gala & Giesy, 1994). Due to carcinogenic and toxic nature of PAHs, many of these compounds are of important concern regarding their adverse impact on ecosystem, as they have the ability to bioaccumulate in marine organisms and resist breaking down (Bedding et al., 1995) (Mill et al., 1981). In the aquatic environment photolysis is the main abiotic degradation process for many PAHs. Photochemical reactions can either be director in direct, direct is due to radiation being absorbed directly by the compound, or indirect (photosensitized), with a chemical transformation being mediated by another light absorbing substance present in the water. There is also a biological transformation of PAHs (Kochany & Maguire, 1994), but this degradation is slow, at least for larger PAHs (Bard, 1994). There have been several studies focusing on the photo degradation of PAHs in water. The direct photo oxidation half-life for different PAHs vary from 2.2 min to 71 h, with more rapid photo degradation for larger PAHs (Kochany & Maguire, 1994). The major photoproducts are quinones, but formation of various hydroxylated derivate has also been observed (David & Boule, 1993). Simultaneous exposure to UV radiation may increase the toxicity of many PAHs (e.g. fluorene, phenanthrene, chrysene, anthracene and pyrene) to a variety of aquatic organisms (Gala & Giesy, 1994). Major hydrocarbons of toxicological interest are benzene, xylene and toluene.

2. 4 Fate of Oil in Sea Water

Oil generally moves upwards to the water surface due to its lower density than sea water Large oil droplets rise more rapidly than smaller droplets and micro-droplets smaller than a certain diameter (b70 μm) have a neutral relative buoyancy and will therefore stay entrained in the water column, unless they coalesce to form larger droplets. Degradation by physical and microbial processes usually occurred in small oil droplets. Oil-dispersing chemicals are usually used to break up oil into smaller droplets and increase the stability of those droplets, thereby controlling the behavior and the fate of the oil. Although only a little portion of the spilled oil reached the shoreline of marine environment, the amount was enough to cause huge contamination of coastal habitats and ecosystems, including many salt marshes, wetlands and estuaries. Compare to oil remaining at sea, landfall of oil is generally considered an unfavorable situation because stranding causes a multitude of new environmental adverse impacts and increase the impacts on fisheries, tourism, and other societal sectors. A wide range of spilled oil is susceptible to oxidation by sunlight. Absorption of light by oil causes the formation of reactive oxygen species that can either be partially oxidized to less volatile residues or completely oxidized to CO2. The important factors controlling the fate, transport and remediation of oil spills, include sediment particle size, concentrations of oil and waterborne organic matter, oil properties, and water salinity (Gong, Y., Zhao, X., Cai, Z., O'Reilly, S.E., Hao, X., Zhao, D., 2014. While fate models indicate that the low molecular weight, primary soluble hydrocarbons dissolved in the deep water were oxidized to CO2 and fixed into microbial biomass. High molecular insoluble nonvolatile hydrocarbons were converted into persistent, partially oxidized compounds.

2. 5 Weathering of Oil

The research Release of oil commence with boundless weathering processes (biotic and abiotic) that transforms its physical and chemical composition. These processes include spreading, dispersion, evaporation, dissolution, dispersion, emulsification, sedimentation, and biodegradation and photo oxidation. Extent of these processes depends on type, characteristics of oil as well as location and condition of environment where the oil spill occurred (National Research Council. 2003). Weathering processes are also responsible for decreasing the amount of oil spilled into water bodies. Fraction of this oil dissolves in seawater and exerts toxic effects on marine environment. The absorbed fraction of spilled oil is sharply toxic to aquatic life (Robotham and Gill, 1989). (National Academy of Sciences, 2003).The main degradation process of PAHs in aquatic environment is photo degradation, which may be direct due to absorption of radiations or indirect by chemical transformation (Kochany & Maguire, 1994). Photo oxidation cause major fraction of oil to convert into oxygenated fraction, which has persisted for years once formed (White and appele) and their degradation is very slow especially for larger PAHs (Bard, 1994). Exposure of oil to sunlight may increase the toxicity of many PAHs to a variety of aquatic organisms (Gala & Giesy, 1994).)

PAHs fraction of oil decreases due to oxygenation. The oxygenated transformation products of petroleum hydrocarbons are likely to have the attributes of persistence, bioaccumulation and toxicity (PBT). The fraction that dominates the mass of weathered oil is large fraction of OxHC and it is formed within days of oil exposed to sun. The oxygenated fraction consists of petroleum derivatives having carbon rich in oxygen. (Christoph Aeppli 2018). OxHCs may be considered as persistent, bio accumulative and toxic keeping in view of their moderate to high hydrophobicity and low aqueous solubility. According to EU REACH regulations, those chemicals are bio accumulative whose bio concentration factor are (BCF) ≥2000 and (BCF) ≥5000 respectively (REACH 2007). BCF is defined as equilibrium partitioning between water and the lipid pool (i.e., membrane plus storage lipid) of an organism. While substances that have toxic effects at concentration higher or equal to 0.1 mg/L concentration in fish are defined as toxic by US EPA. When oxygen is added to the normal chain hydrocarbons, their bioaccumulation potential decreases with respect to their parent compounds while their solubility increases because of increase in lipid solubility of oxygenated compounds. This makes them bioavailable than their parent PAHs.

As weathering process is the main driver to change the composition of oil, these changes cause lethal and acute toxicities to the marine organisms (Liv-Guri Faksness.) Larger fraction of oxygenated HCs having higher molecular weight are resistant to biodegradation and become recalcitrant, so photochemical transformation is the initial step in their degradation. (Sarah et al, in 2014) studied photo degradation of oil from deep water horizon oil spill. Research was conducted in the presence of catalyst (TiO2) to obtain a better understanding of environmental behavior and also in the absence of photo catalyst to increase the process of photo degradation Study has been conducted using a mercury lamp for the photo degradation followed by analysis of oil using GC- FID. The key finding of their research indicates that sunlight plays an important role in degradation of large, bio refractory PAHs and the use of photo catalyst enhances the degradation of PAHs, especially for smaller PAHs. Sunlight is responsible for the increased toxicity of water due to release of toxic water soluble photoproducts. In 2016 B.Echols et al conducted a chronic toxicity test on Moconda oils collected from fields and source during the 2010 deep water horizon (DWH) incident, using standard test species mysids and inland silversides to evaluate changes in survival and growth during daily renewal tests. The results showed increased sensitivity to low energy water accommodated fractions (WAFs), with growth and survival decreased with increasing oil loading rate (Menidia beryllina). Irradiation of oil to the surface of water causes addition of oxygen to the carbon chain and formation of superoxide. Further reaction to superoxide resulted in the formation of hydroxyl radicals. These radicals play an important role in the degradation and oxygenation of compounds in oil. Phoebe et al, in 2014 studied rate of hydroxyl radical in artificially spilled irradiated oil. TiO2 Nano particles were used to identify change in the rate of formation of Hydroxyl radical. Competition kinetics approach was also used to measure the steady state concentration of Hydroxyl radical. Results reveal

TiO2 could be used to speed oil degradation by decreasing barriers in transport of these radicals into aqueous layer. A research has been conducted by Taleet saeed et al, in 2009 to recognize the photo dynamic effect of petroleum contaminants in sea water of Kuwait by environmental conditions such as temperature, light, intensity, oxygen level and sensitizer in laboratory and found all the factors to play a significant role in increasing the degradation of PAHS in oil.. Similarly, in 2003 Fasnacht and Blough have identified the consequences of photolytic process on a variety of pure hydrocarbons under solar and simulated solar conditions. (Fasnacht, M.P., Blough, N.V, 2003). Weathered oil due to sunlight irradiation degrade large, aromatic HC molecules, generating oxidized species that are more water soluble than the parent compounds. Ray et al. (2014) and Radović et al. (2014) investigated the complex formation of oxygenated transformation products when WAFs of oil are exposed to sunlight. Phoebe et al,in 2014 assessed the impact of sunlight on oil fate by mixing oil and irradiating it with simulated light, characterized using FT-ICR MS. Results showed high concentration of oxygen classes, as the oil concentration decreased after irradiation, so the concentration of Pynidinic nitrogen decreased and abundance in NiOx increased. Ray and Tarr (2014) further studied the key role of hydroxyl radical production in the oil photo degradation process and how the adding of TiO2 photo catalysts can speed up oil degradation. Yang Guipeny et al, studied the photochemical reactions due to exposure of oil to sun in oceans and factor which influence the photo degradation process by using natural sunlight and artificial sun light using mercury lamp . Results showed that not only sunlight affects the rate of photochemical reactions but other factors are also involved. Decomposition of oil using photo catalyst and UV lamp showed that the absence of oxygen decreased the decomposition of organic compounds in oil. (R.James Berry). Toxicity of oil to species at different levels was being studied by (Liv et, al in 2015). Chemical characterization of two source oil and five field oil were being collected from deep water Horizon incident to identify changes that occurred in the chemical composition due to weathering of oil. WAFs of both source and field oil shows that toxicity is higher in WAFs form non weathered oil. The effects of crude oils and petroleum products in the marine environment have been extensively studied since the 1970s. However, the wide variety of biological and chemical analytical methods employed has produced a diverse collection of data which does not easily lend itself to cross-comparison. OxHCs are ignored in recent risk assessment models, their fate, behavior and toxicities not well- known. They are ignored in oil spill risk assessments because of the lack of information about the levels, molecular structures and toxicities of transformation products. (Nabi et. al, 2018) this fraction was not chemically and biologically degraded. (Ryerson, T. B) It persisted for at least six years challenging the current risk or damage assessment-based approaches for hydrocarbons (Ward). There is a contradiction between recalcitrance and biodegradability of OxHCs (Aeppli 2018).

2. 6 Effects of Oil Spills on Animals

Upon spill, oil spreads out in a thin layer. Volatiles evaporate while other dissolves in water. UV radiations and wave actions break down oil components while some become persistent depending on the oil composition. Physical parameters including temperature, light intensity and water turbulence also play an important role in oil degradation and persistency. Fishes, mollusks, and small crustaceans are greatly affected by oil droplets. They entrap and kill them. Bird may also inhale them or get it stuck on their bodies hindering their ability to fly. (Arne Jernelov).

2. 7 Passive Sampling Phases as Alternatives to Fish Testing

According to European Union Regulation body REACH more than 21,000 chemicals are registered in 2019 which are being used in market. Global production of chemicals has increased from 1 million tons to 400 million tons in a century. European Union (EU), 2001). The hazardous effect of these chemicals on biota and abiota is of great concern.

D. Mackay. The large number of existing chemicals requires proper risk assessment before use. According to regulatory bodies such as European Union REACH (Registration, Evaluation and Authorization of Chemicals), legislation requires risk assessments and toxicological hazard assessment for all new and existing chemicals. (A.P. Worth)

Under REACH program, large number of animals is used for the risk assessment of these chemicals. Conventional methods of testing toxicity of chemicals on animals are laborious, costly and time consuming (McGrath and Li, 2008, Rubinstein, 2006). Use of chemicals for the testing of these abundant chemicals adversely affects animals. Many mammals such as rats and mice are used as models for drugs and chemicals toxicity assessments. (S.kuriyama) However, a considerable reduction in animal use could be obtained if these techniques are applied more intensively. If the use of alternative methods are not accepted by regulatory authorities 3.9 million additional animals could potentially be used as a consequence of the introduction to REACH.

Approximately 1.9 million test animals would be saved if alternative techniques are accepted and used. These savings can be obtained by introducing and accepting methods that are, to a large extent, available today. (Katinka van der Jagt). Using new scientifically-sound techniques are essential for obtaining and interpreting relevant and reliable data which increase the effectiveness of risk assessment processes. Alternative approaches decrease the use of animals for testing chemicals and increase the risk assessment processes (Bradbury et al., 2004). Lots of experiments are conducted and theoretical models are developed to predict permeation of chemicals into animals. Among the various models, PDMS (polydimethylsiloxane) membranes are of particular interest due to the lipoidal PDMS has lipoidal nature to mimic the stratum corneum, the rate limiting barrier of the skin (Wasdo et al., 2008). Also PDMS membranes are favorable for their reproducible composition and thickness, simplicity of use and cost effectiveness. Many studies have been carried out to investigate the drug diffusion or permeation across PDMS membranes (Moss et al., 2006; Sloan et al., 2013; Wasdo et al., 2009. Many studies have been carried out to investigate the drug diffusion or permeation across PDMS membranes

(Moss et al., 2006; Sloan et al., 2013; Wasdo et al., 2009). Mode of action of every chemical includes one of the following eight mode of actions. Base – line narcosis or narcosis i, polar narcosis or narcosis ii, ester narcosis, oxidative narcosis, oxidative phosphorylation uncoupling, respiratory inhibition, electrophile/proelectrophile, reactivity, AChE inhibition, and CNs seizure responses (Christine 2018)

Among test organisms, fish is an important freshwater species in aquatic food webs that has been used world-wide for many years as a representative test species for eco-toxicological evaluation of industrial chemicals. (C. Barata,) (Y.Kamaya)

The Goal of this objective is that we can use PDMS as an alternative method instead of animals.

In this research, the uptake of chemicals across the silicon membranes was used. PDMS is significantly correlated with that of fish (fatehead minnow).

Chapter 3

Material and Methods

3. 1 Material and Methods

The toxicity of petroleum products in water is due to its water soluble aromatic hydrocarbons. Compounds with low molecular weight do not persist in the environment as they are volatile while higher molecular weight compounds are persistent and have long term impact on environment (Joy, A). Acute toxicity is based on the hypothesis that the aqueous concentration for a toxic endpoint, such as median lethal concentration (LC50) can be predicted from the critical body in the target lipid of an organism CM which can be calculated from the target lipid-water partition coefficient $K_{\text{M-W}}$

$$LC50 = Km{-}w \times Cmembrane$$

$$Cmembrane = 100 \text{ mmol}/kglipid$$

$$-\log LC50 = 1 + \log Km{-}w$$

The hypothesis is if we replace membrane with PDMS, a widely used passive sampler then

$$\text{Log } LC_{50} = 1 + \log K_{m\text{-}w}$$

$$\downarrow$$

$$\text{- }\log LC_{50} = 1 + \log K_{PDMS\text{-}w}$$

The uptake of chemical will be similar and we are able to capture the toxicity in a good way. The toxicity of petroleum products in water is due to its water soluble aromatic hydrocarbons

3. 2 Preparation of Artificial Sea Water

Take 1 liter of distilled water in a volumetric flask, measure and add following regents and adjust PH 7.8 and temperature after that autoclave it and store it at 4˚C.

Table 3. 1: Sea water Preparation using NOVAA protocols

Regents	Quantity
NaCl	17.52g
KCl	0.74g
CaCl2.H2O	0.14g
MgSO4(H2O)	12.32g
(NH4)HPO4	0.13g

Table 3. 2: Experimental Design for toxicity Assays

Time exposure	Experimental setups 1+2(1setup with 2 replicates	Number of female copepods	Number of Dilutions 100%,75%, 50%, 25%	Volume of Artificial sea water and oil for WAF	Volume of oil Fraction of oil and water
Dark control	3 vials	30	4	499ml+1ml oil	500ml
5min	3 vials	30	4	499ml+1ml oil	500ml
10min	3vials	30	4	499ml+1ml oil	500ml
15min	3vials	30	4	499ml+1ml oil	500ml
20min	3vials	30	4	499ml+1ml oil	500ml
25min	3vials	30	4	499ml+1ml oil	500ml
30min	3vials	30	4	499ml+1ml oil	500ml

3. 3 WAF preparation

In this paper, these guidelines were developed to standardize WAF preparation, laboratory exposures to aquatic organisms, and analytical chemistry measurements used to determine the acute toxicity of the water soluble components in the oil (Aurand and Coelho, 1996).

Water accommodated fractions (WAF) were generated according to the standardized protocol established by the Chemical Response to Oil Spills: Ecological Research Forum (CROSERF). Briefly, WAFs were prepared at room temperature in 500 ml sample bottles with 20% headspace by volume using artificial seawater made using NOAA protocols (with salinity maintained at 32- 33 parts-per-thousand). 999 ml of autoclaved ASW was taken in 1 liter sample bottle to which 1 ml of light jet oil was added. The lid was tightly closed. The sample bottle was then left in automatic shaker for 24 hours followed by 2 hours settling time after it was transferred to the beaker. Oil makes an undissolved layer on seawater. The oil phase was pipetted out using a 5ml glass pipette until a thin sheen on water disappeared. 4 dilutions (100%, 75%, 50% and 25%) of this WAF was prepared with autoclaved seawater in which 30 female copepods being segregated from sampled water were drawn in each and covered with Aluminum foil having small pores for oxygen exchange to avoid contamination. The WAF with test organisms was left for 24 hours (12 hours light followed by 8 hours dark) to count LC50 values.

Table 3. 3: Dilution Factors of each experimental group

Dilution #1	Dilution #2	Dilution #3	Dilution #4
100% WAF	75% WAF	50% WAF	25% WAF
100ml mixed oil +100ml sea water	150ml mixed oil +50ml sea water	100ml mixed oil +100ml sea water	50ml mixed oil +150ml sea water

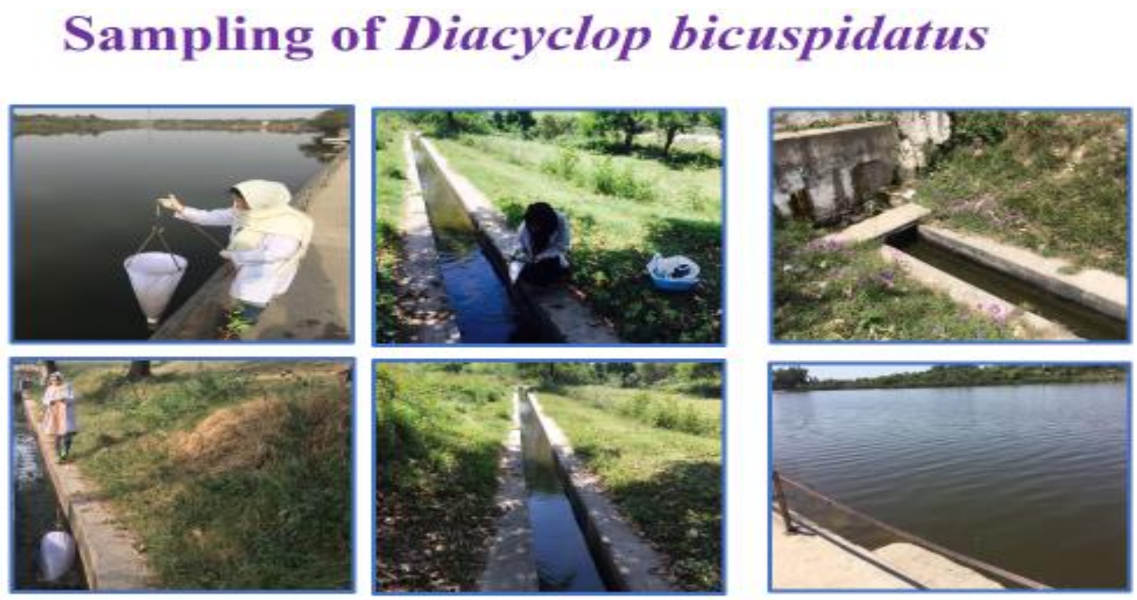
3. 4 Experimental Animals

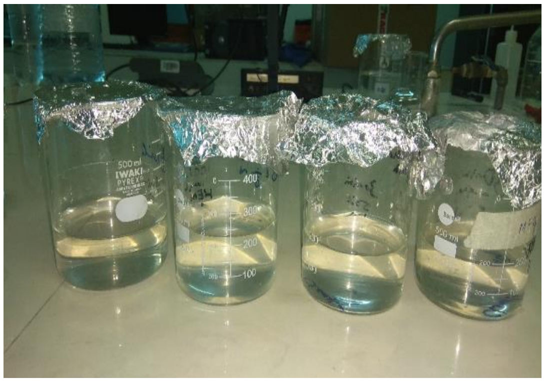
Copepods are an important source of food in marine food web. Herbivorous copepods of the species (*Diacyclop bicuspidatus*) were sampled from an agricultural pond Mesiroit Dam, Rawalpindi in March 2019. Organisms were collected using net hauls (200 um mesh). The mean temperature of the upper 100 m water was recorded. PH was measured with pH meter, salinity was measured using conductivity meter and DO was measured using DO meter. They were then transported to IESE, NUST in a thermostable container. Upon arrival, the organisms in the containers were transferred to plastic tanks after sieving with a 200 um mesh and left for egg production and growth of organisms for one week. The tanks were provided throughout the rearing period with indigenous algae and Eulidia plant which is found in downstream spill way of Misiriot dam where maximum richness of copepods was recorded.

Sampling of zooplanktons was carried out first time on 11th November 2018 from three stations. Upstream, downstream and from surface of water body (station 1, 2, and 3 respectively). Population of copepods was low during the first sampling. Different species of zooplanktons were collected. Among them the predominant copepods were Daphnia, calanoids and Cyclops. In this research, work was done on *Diacyclop bicuspidatus*, species of class Cyclops. The next sampling was conducted on 1st, February 2019 when the population of organisms was observed to be still low; population of species *Diacyclop bicuspidatus* was also low. After 20th February, population of all zooplanktons started to increase because of increase in temperature. Again a sampling was conducted in the last week of February where the population of the organisms was observed to be rising. But the sampling done in March and on 1st and 14th April 2019 showed maximum population density. The overall richness of the test organisms is maximum in spring and summer. Richness of females was high in all samplings while males were extremely rare. Procedure of sampling and recording of physical properties of sampling water was done in similar manner as done the first time. Test organisms that were chosen for the experiments were all females.

The test organisms were exposed to a dilution series of the water from the WAF experiments in order to determine the effect concentrations (EC50 and LC50).

Figure 3. 1: Sampling on field.





3. 5 Type of Oil Used for This Research

Type of crude oil that was used for this research is JP-1. JP-1 oil is a complex mixture produced by fractional distillation of crude oil. The actual composition of jet oil depends upon the source of crude oil (physical and chemical properties of military fuels). JP-1 has a carbon number between 8 and 16 carbon atoms per molecule. It has a low freezing point of about -40˚C (-40˚F) and its flash point is 30˚C. Density of jet oil at 15˚C is 0.804kg/l. Specific energy is 42.80 MJ/Kg. JP-1 oil was chosen for this research to assess the effect of weathered small fraction hydrocarbons on the test organisms(zooplanktons) in marine water bodies after oil spills.
(https://en.wikipedia.org/wiki/Jet fuel)

Table 3. 4: Properties of JP-1 oil given by Attock oil refinery are as given below

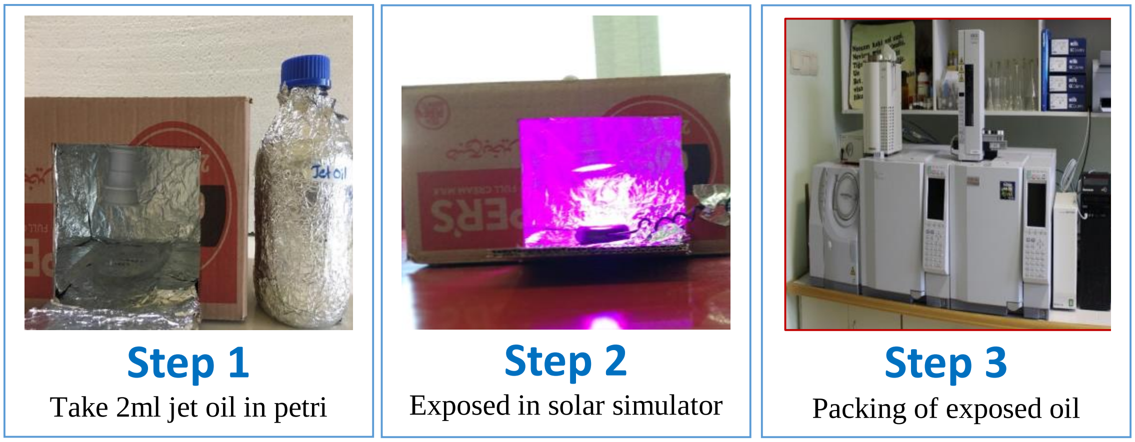
Properties	Unit	Specification	Min- max
Appearance	-	clear and bright	-
Color	-	Report	-
Particulate contamination	mg/l	1	Max
Boiling Range	centigrade		150-225
Carbon Chain			C9-C14
Flash Point	℃	38	-
Density at 15ºC	Kg/m3	775.0 min to 840.0 max	-
Freezing Point	℃	-47	Max.
Viscosity at -20ºC	cSt	8	Max.
Specific Energy, net	MJ/Kg	42.8	Min.
Electrical Conductivity	pS/m	50 min to 600 max	-

3.5.1 Experimental steps for phase 1

3.5.1.1 Photo irradiation of jet oil

The solar simulator used in this study to expose oil was made using grow light having an optical spectrum and intensity slightly higher than that of sun. To standardize the instrument, a LUX meter was used at the solar laboratory CASEN that measured the light intensity of the closed solar simulator at different positions and heights. Solar simulator is covered internally by Aluminum foil to obtain maximum reflection. Oil samples were placed in a petri dish and exposed to light in the solar simulator for different times (2, 5, 10, 15, 20, 25 and 30 hours). The oil was collected after each irradiation and collected in amber glass bottles. Samples were then analyzed in GC/MS for characterization of newly formed compounds. A dark control without irradiation was also performed in the same manner. 20% of the exposed oil was reduced after irradiation. Approximately 4 hours of irradiation in the simulator was equivalent to one day of sunlight, on average. Surface temperature was measured carefully with a thermocouple after every one hour of irradiation, showed an increase in temperature, which indicated that irradiation of the oil caused an increase in the surface temperature. Dark control was also observed to loss of C14–C17 alkanes

Figure 3. 2: Step of photo irradiation.



Step 1
Take 2ml jet oil in petri

Step 2
Exposed in solar simulator

Step 3
Packing of exposed oil

3.5.1.2 GC-MS protocols for chemical characterization of jet oil

OxHCs concentrations were largely determined using GC-MS following the EPA procedure 8270d. A gas chromatograph (ShimadzuGC-14A) coupled to a mass spectrometer (Shimadzu GCMS-QP2000) was used to identify degradation products operating at a pressure of 10 psi and a flow of 30 cm/s. A split less injection was used.
Temperature of 250 ◦C was set for both the injector and the detector. The column was kept at 40◦C for 2 min after injection and the temperature was then increased by 10◦C every minute until a maximum temperature of 280 ◦C was reached. This temperature was kept for 5 minutes before the column was conditioned at 320◦C for 5 min. These conditions resulted in baseline separation of all the compounds to be analyzed

3.5.2 Experimental steps for phase 2

3.5.2.1 Photochemical exposure of jet oil for lethal and sun-lethal toxicity

Photoreaction incubations were made of jet oil in a petri dish that has thoroughly been washed with distilled water and then autoclaved. The irradiation source used during the photo oxidation of oil in this research was solar simulator. The source of solar was full spectrum grow light of 220V. The time has been set at 5, 10, 15, 20, 25 and 30h. The emitted UV radiation was comparable to solar radiation on a clear summer day in Islamabad and dark controls were treated identically, apart from being wrapped in aluminum foil and put in dark for same period of time.

3.5.2.2 Toxicity Assays

3.5.2.2.1 Methodology for Lethal Toxicity

In this research the lethal effect of OxHCs was tested by exposing copepods, *Diacyclop bicuspidatus*, to aqueous fractions from the WAF. Thirty individual adult female Diacyclop for along with three replicates and one control groups in each setup were sorted and exposed to the whole WAF at 100%, 75%, 50% and 25% concentrations at room

temperature (put 30 female Diacyclops in each dilution). A 0% concentration (i.e., pure seawater) was also included as a control in which 30 female copepods were added.
This design was replicated for both polar and nonpolar fractions of the WAF. The copepods were exposed for 24 hours (16 hours light and 8 hours dark period). Dead copepods were counted, and morality was calculated relative to the control group.

3.5.2.2.2 Methodology for sub - lethal toxicity

The concentration to be tested for the sub lethal effects of OxHCs were chosen based on the results from the lethal toxicity experiment. New batches of *Diacyclop bicuspidatus* were exposed to the highest concentration of the each of the WAF fractions at which no lethal effect was seen in order to find out the sub lethal effects such as respiration rate and swimming behavior.

3.5.2.2.3 Dissolve Oxygen

Oxygen consumption by the copepods was measured during 24-hour exposure to different sub-lethal concentrations. The organisms were sorted, placed in narrow mounted glass bottles, after making 5ml, 4ml, 3ml, 2ml and 1ml concentrations of WAF. The beakers were then cover with Aluminum foil and left for 24 hours (16 hours light and 8 hours dark). After 24 hours, the organisms were drawn out and put in fresh seawater in a measuring cylinder. An oxygen electrode is dip into the measuring cylinder in such a way that no air from the atmosphere can enter the measuring cylinder. A control is kept with each experimental group. The result of experimental unit containing one or more dead animals was rejected.

3.5.2.2.4 Swimming Behavior

The swimming behavior of the copepods in sub lethal concentrations of WAF was observed. The purpose was to determine whether photo oxidized WAF alters the pattern of swimming behavior; and if how a further response would be like. It was predicted that sensitive indicator of the animal’s responses to their environment includes their swimming behavior because, especially in herbivorous copepods, routine locomotion depends on their feeding, (Gauld, 1966, koehl and stickler, 1981; stickler, 1982, Zaret, 1080)

Sub lethal concentrations for each time exposure were prepared and five female and five male copepods for each jar were sorted and put into 5ml, 4ml, 3ml, 2ml, and 1ml concentrations of WAF. Animals were covered with Aluminum foil with pores for air movement and left for 24 hours (16h light and 8h dark). After 24 hours, the organisms were drawn out and transferred into vials containing uncontaminated seawater. A camera was set up outside the vial, monitoring the swimming of the copepod for 10 minutes. The recording was used to track behaviors of the animal. This was repeated with one male and one female copepods of each dilution to produce individual copepod recordings. This procedure was replicated for the polar fraction and nonpolar fraction of the WAF

3. 6 Third phase of This Research

The goal of this objective is that can we use PDMS a passive sampling phase as an alternative method instead of animals.

In this research, the uptake of chemicals across the silicon membrane phases were used and found that PDMS is significantly correlated with that of fish (fate head minnow)

Chapter 4

Results and Discussions

4. 1 Water Characteristics

Different Dissolved oxygen, pH, temperature and salinity of sampling water body were recorded to be 8.5 ± 0.07 ppm, 7.75 ± 0.02, 20 ± 0.2 C and 32 psi, respectively.
Data on the lethal toxicity against time of the copepods were statistically analyzed using statistical tools to compare between the exposed and control groups in the lethal and sub-lethal toxicity test.

4. 2 Interpretation of GC-MS results

The chromatograms are quite complex. The main difference between dark and exposed samples is that the exposed samples are moving towards higher retention time which shows increase in molecular weight of increased transformation products due to addition of oxygen. This actually confirms that the sunlight is inducing the photo oxidation in the oil compounds

Figure 4. 1: Dark control

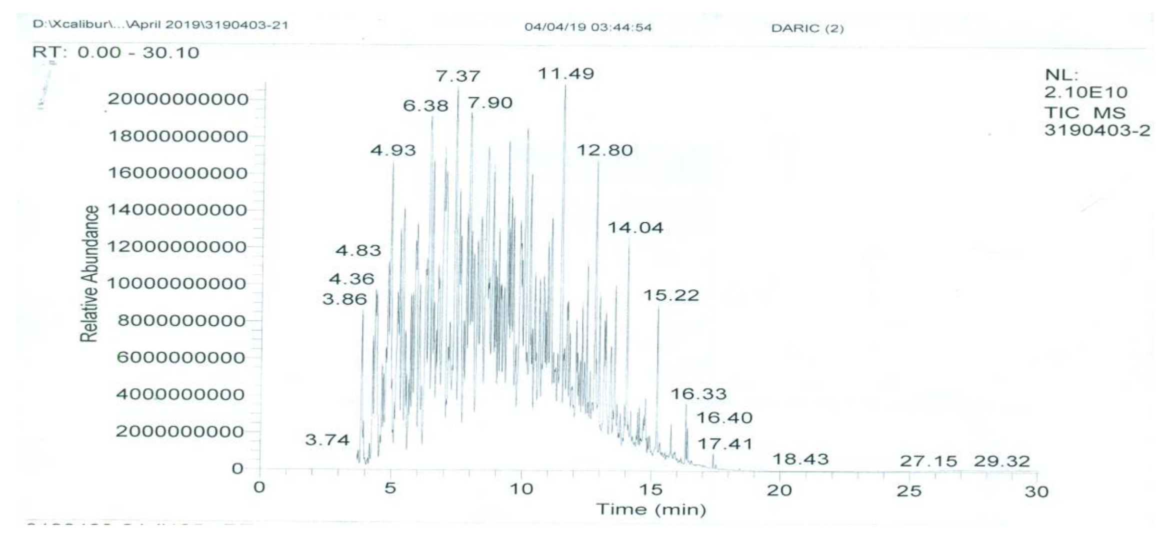
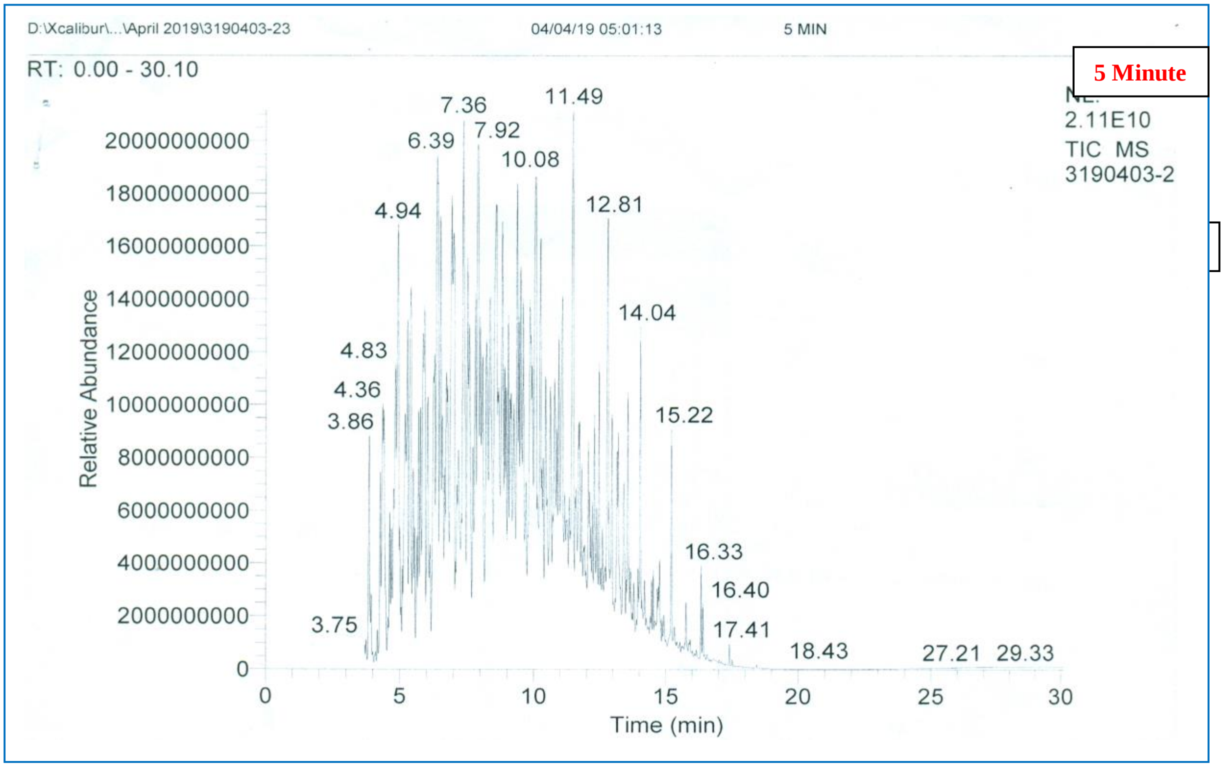
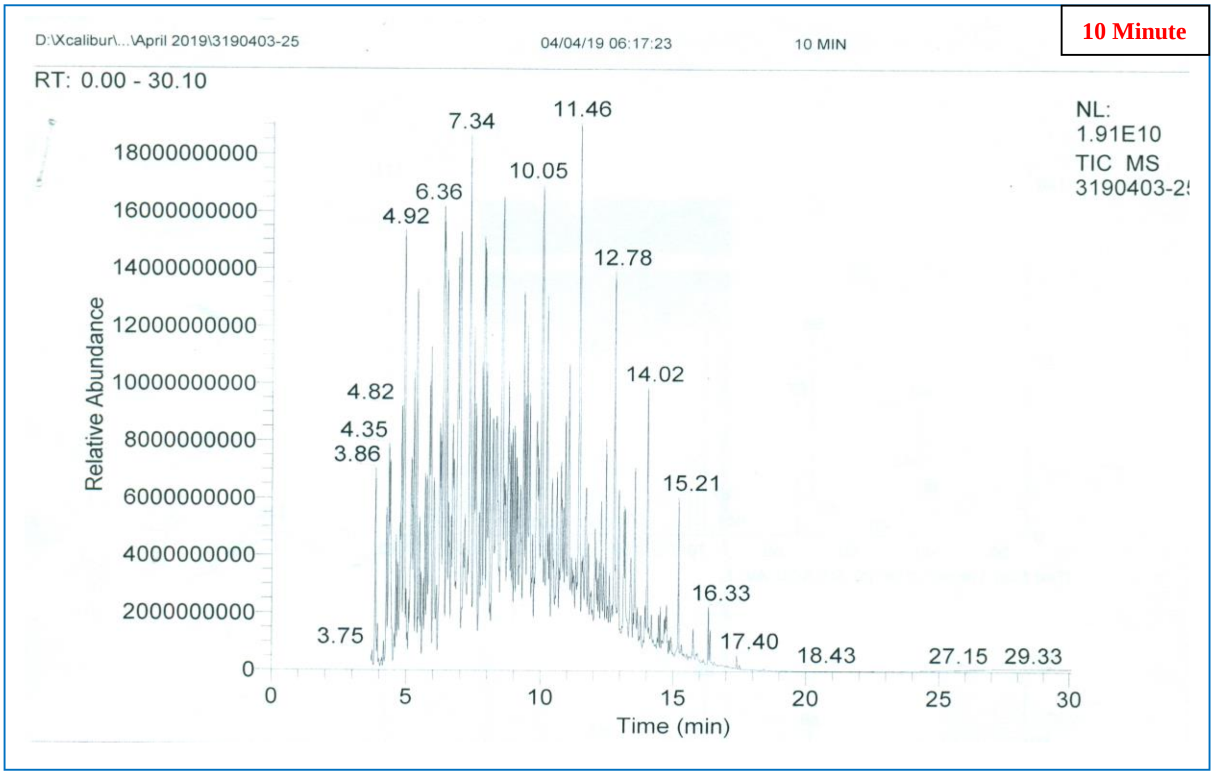


Figure 4. 2: 5 & 10 minute exposure to simulated sunlight

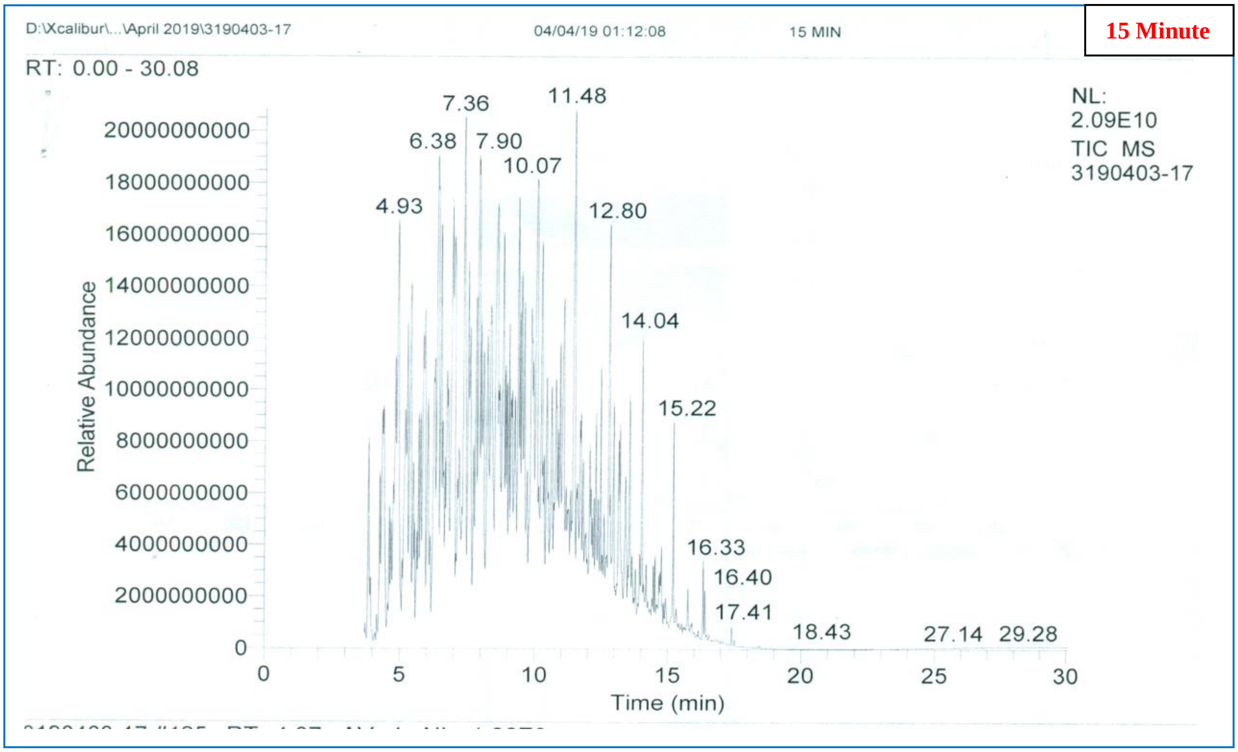


5 Minute



10 Minute

Figure 4. 3: 15 & 20 minute exposure to simulated sunlight



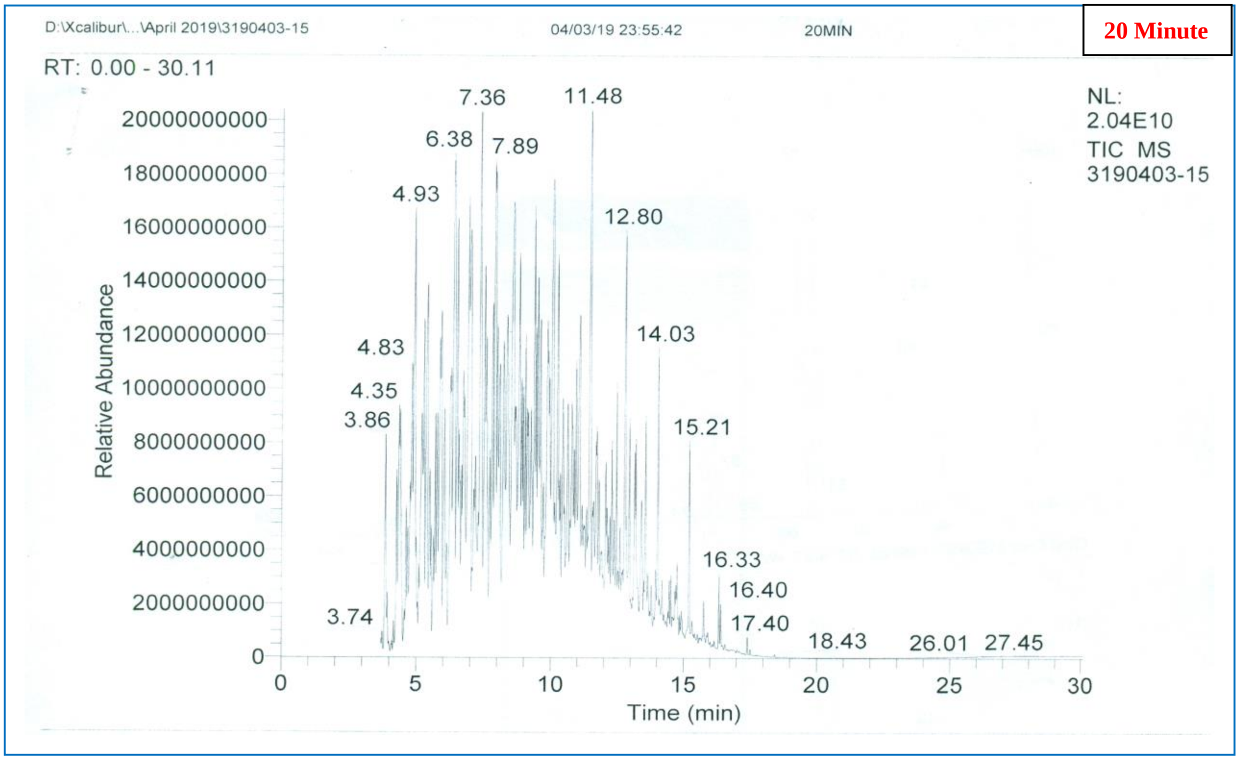
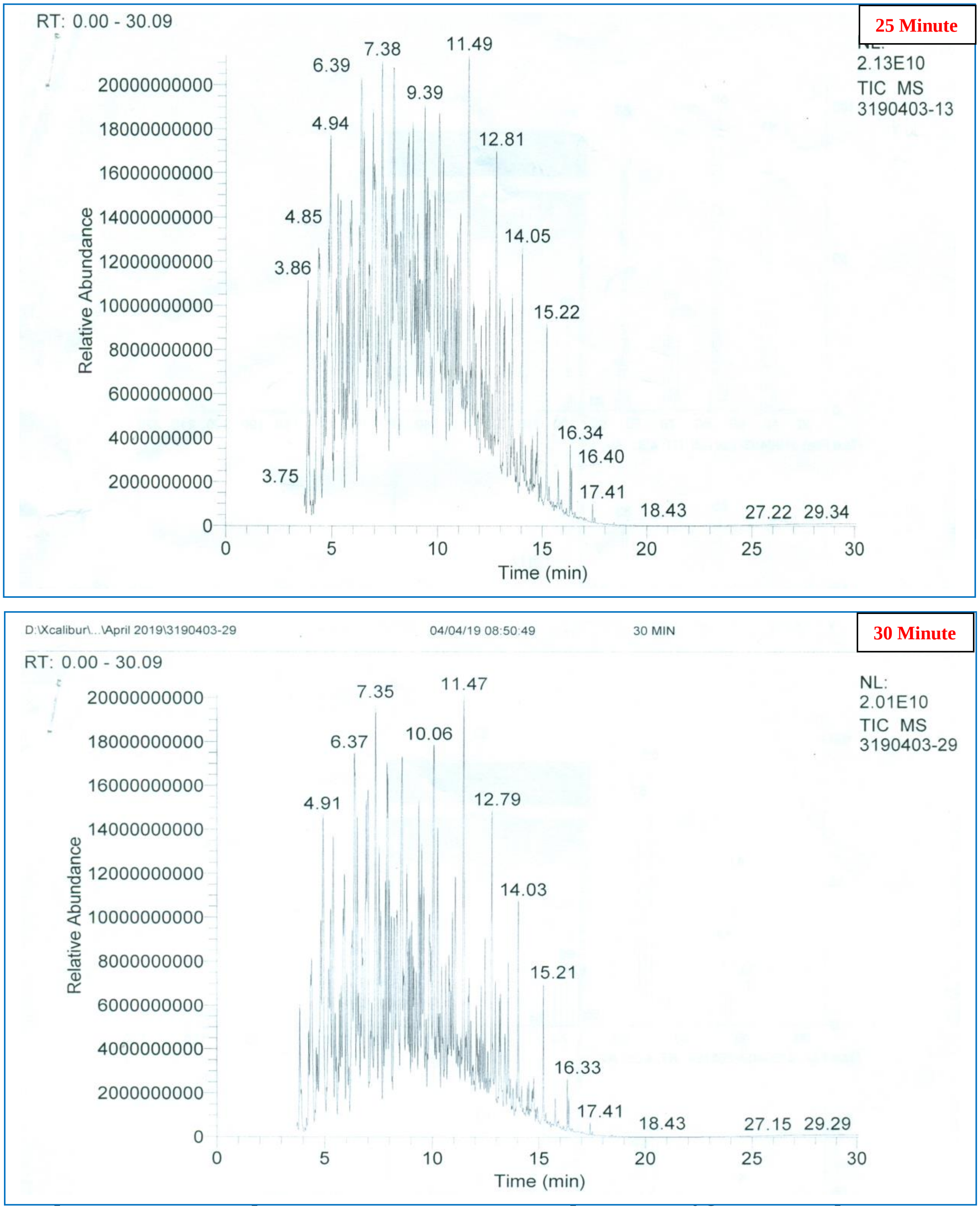
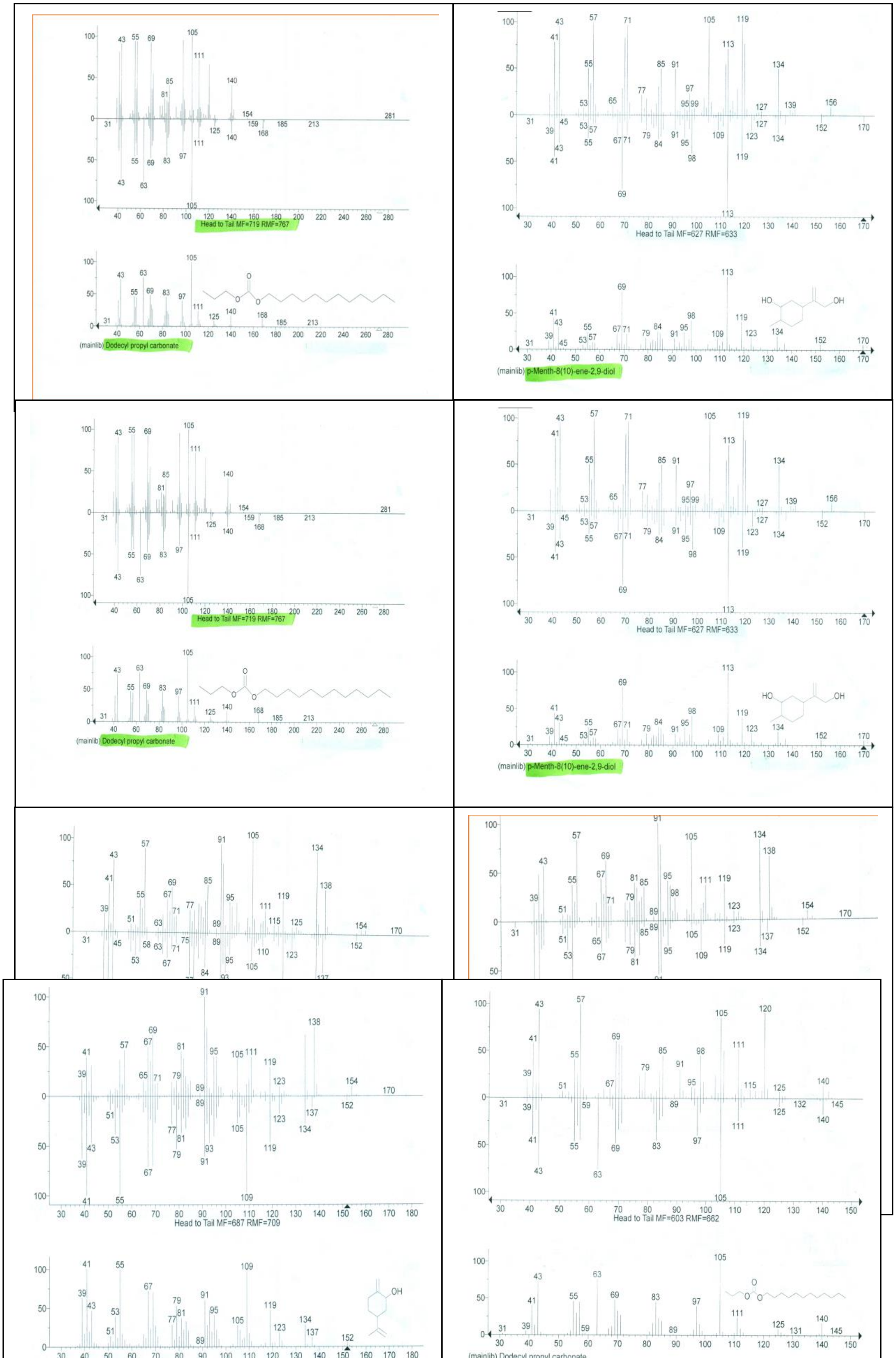


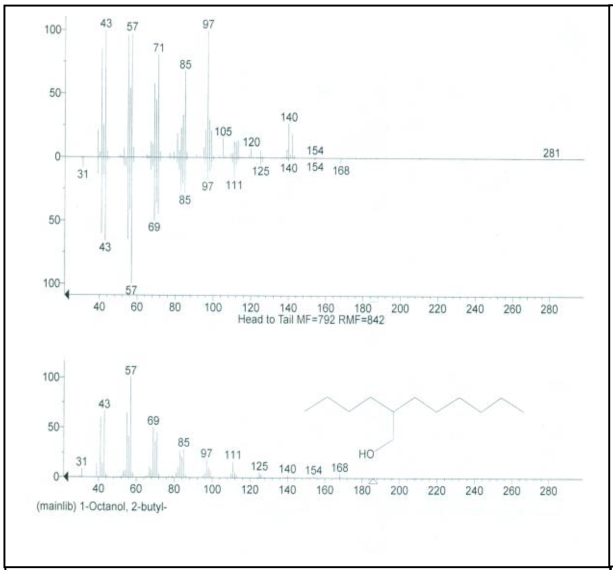
Figure 4. 4: 25 & 30 minute exposure to simulated sunlight

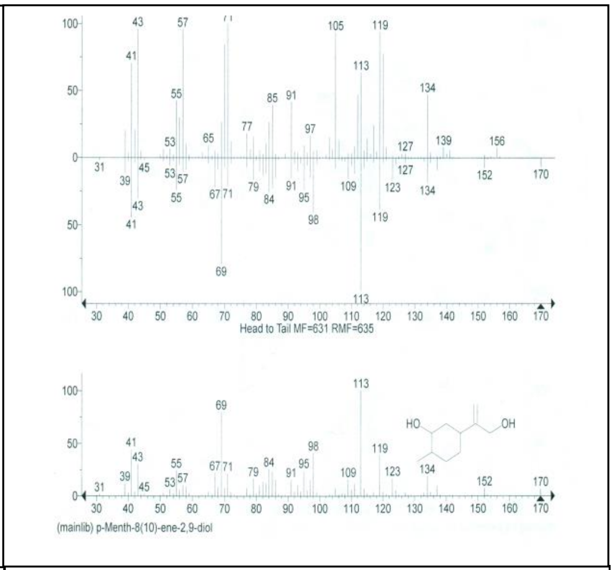


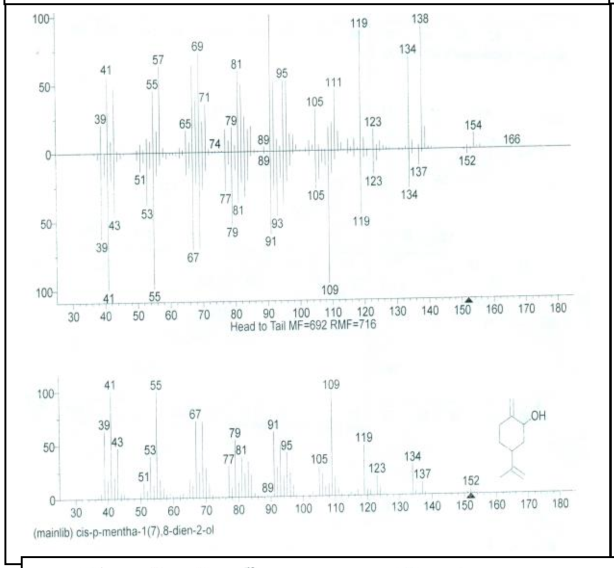
Their resemblance score with the reference spectrums in the NIST library are given below.

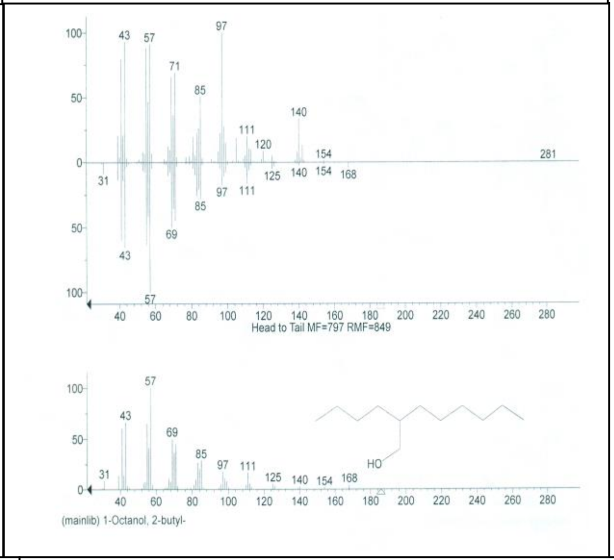
Figure 4. 5: Identification of transformation products

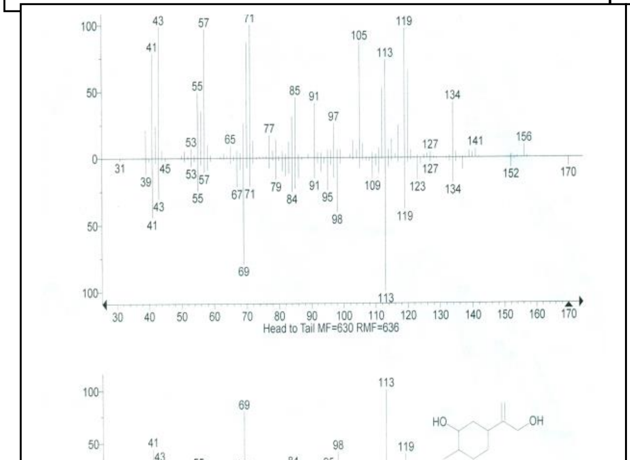



Head to Tail MF=792 RMF=842
HO
(mainlib) 1-Octanol, 2-butyl-


Head to Tail MF=631 RMF=635
HO
OH
(mainlib) p-Menth-8(10)-ene-2,9-diol


Head to Tail MF=692 RMF=716
OH
(mainlib) cis-p-mentha-1(7),8-dien-2-ol


Head to Tail MF=797 RMF=849
HO
(mainlib) 1-Octanol, 2-butyl-


Head to Tail MF=630 RMF=636
HO
OH

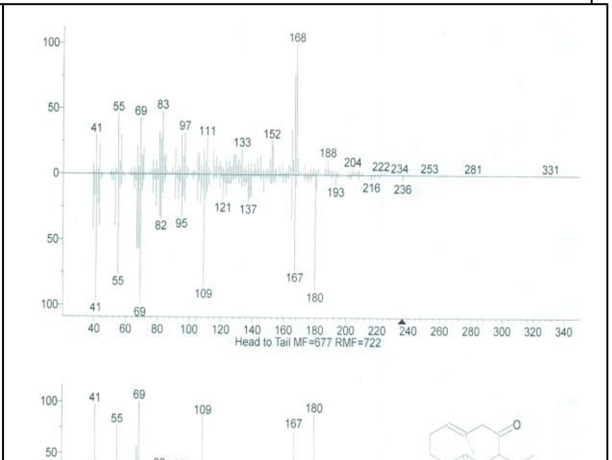

Head to Tail MF=677 RMF=722
O

Table 4. 1: Identification of transformation products in Photo irradiated GC-MS samples

Transformation Product	Compounds
Dodecyl Carbonate	1 octanol,2- butyl
Cis-p-Mentha-2-8-dien-1-ol	7-Methyl-z- tetradecen-1-ol acetate
Neocurdione	Cis-p-menthe 1(7),8-dien-2-ol
3(10)-caren-4-ol	Trans-Verbenyl caprate
Cis-p-menthe- 1(7),8-dien-2-ol	1-Octanal-2- butyl
Dodecyl Propyl carbonate	P-Mentha- 8(10)-ene-2,9-diol
4a,10a-Methanophen anthren-9β-ol,	2,5-Octadecadiynoic acid

4. 3 Results of lethal toxicity

Data on the lethal toxicity against exposure time of the copepods were statistically analyzed using statistical tools to compare between the simulated sunlight exposed samples and control group samples in the toxicity test. Experiments were conducted to get the LC 50 (median survival time) values for all of the concentrations and oil exposed time period. The copepods tested were at their mature stage. Study showed that the copepods exposed for longer period of time experienced more lethality and showed high sub-lethal effects compared to the ones exposed for short period of time in simulated sunlight. After 24 hours, dead Diacyclops were counted using microscope and magnifying glass. The dead Diacyclops were recognized by their faded body color, lack of movement of appendages, no response to touch with glass pipette and their often sinking to bottom of the beaker.

Results of lethal toxicity assays shows increase in lethality as a function of exposure to sunlight. The simulated sunlit samples show higher toxicity than the dark control. The longer the oil samples were exposed to the simulated sunlight, the more toxic it made the WAF. For both dark and sunlit samples, the higher the loading of WAF, the higher is the toxicity.

Figure 4. 6: The 10 min plot shows higher toxicity than 5 min exposure.

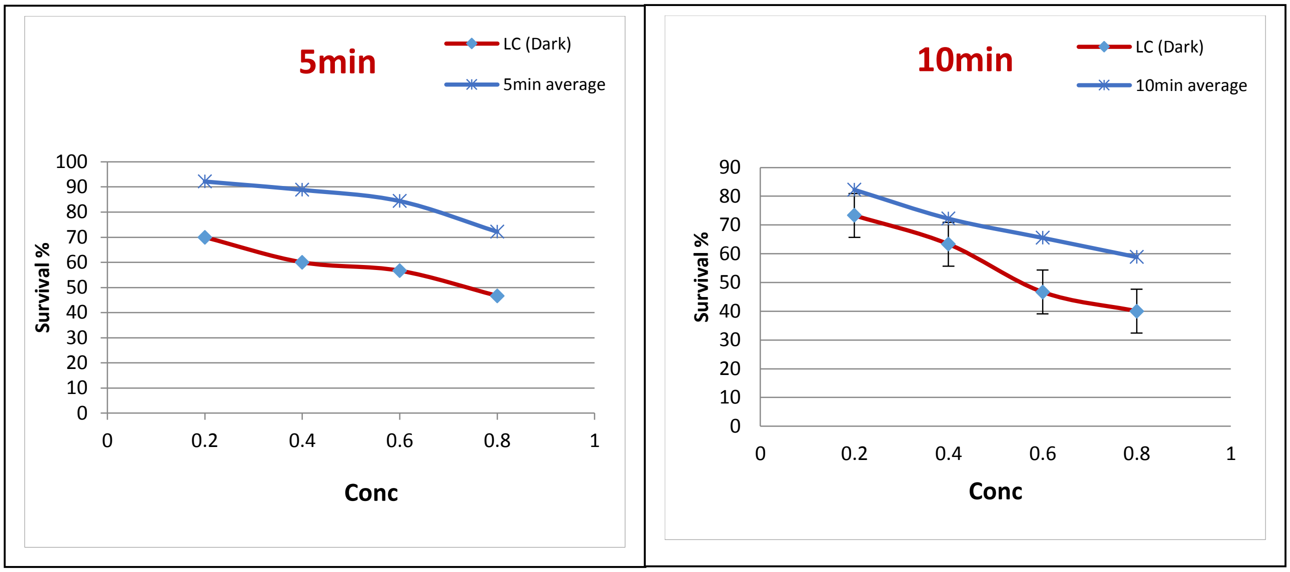
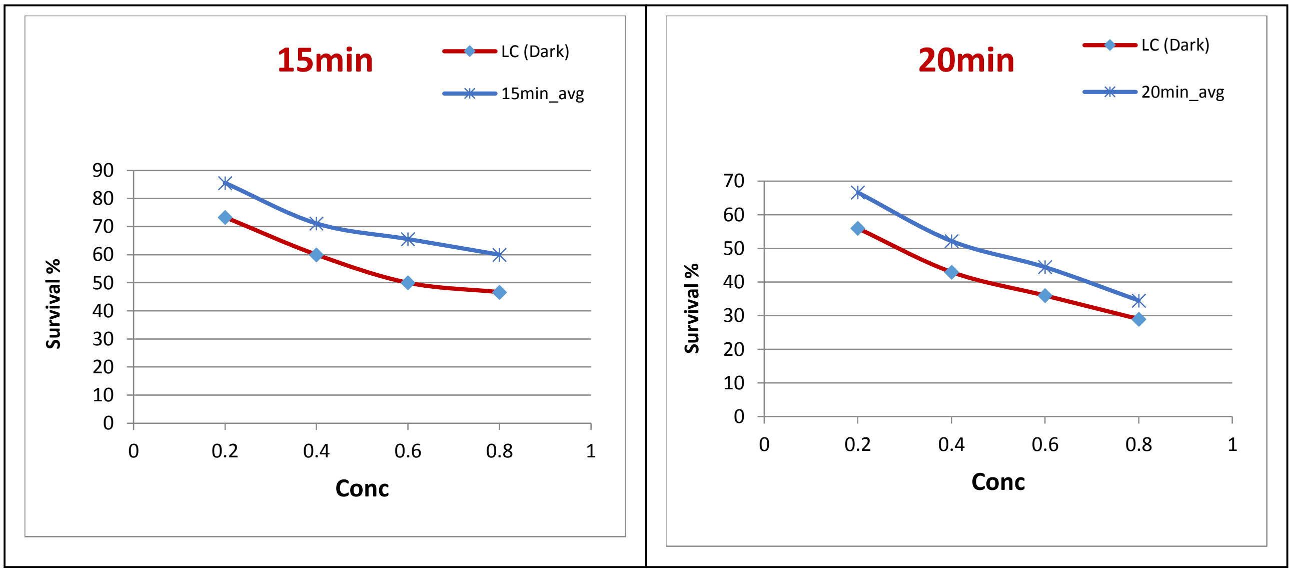
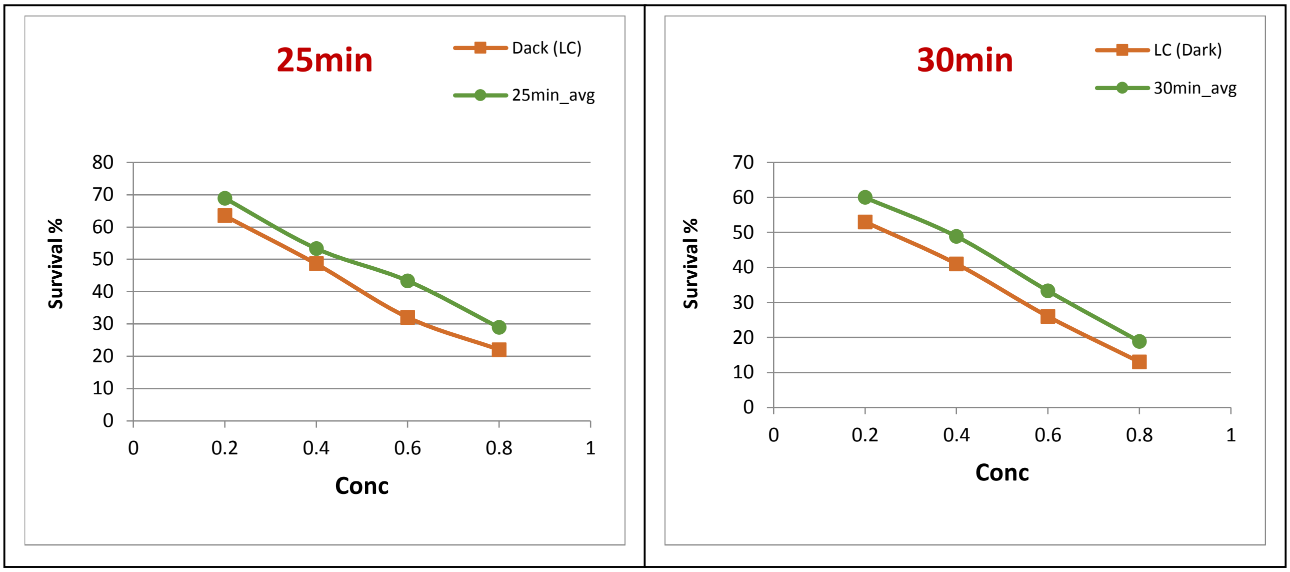


Figure 4. 7: The 15 min plot shows higher toxicity than 20 min exposure.





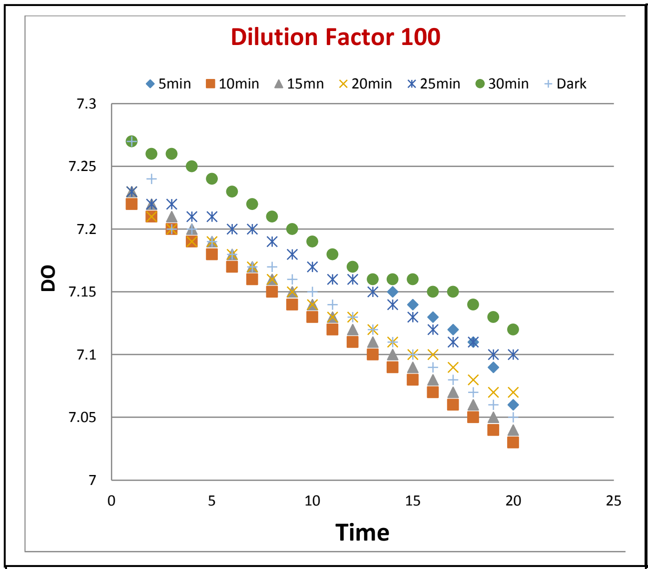
4. 4 Results for Sub Lethal Toxicity

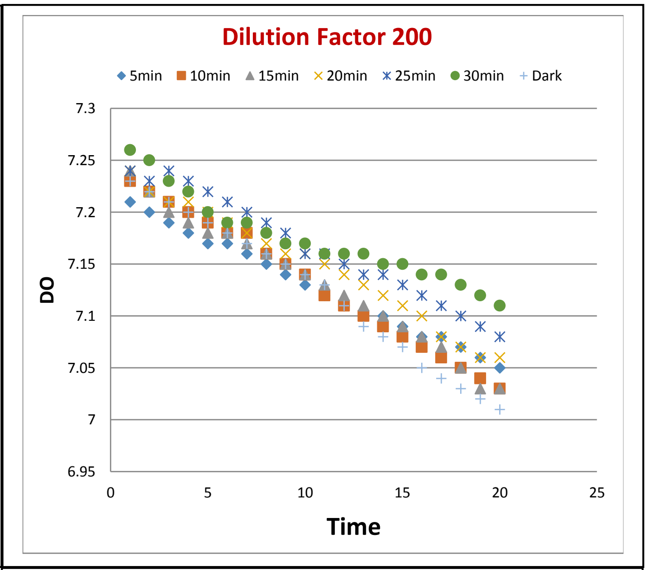
4.4. 1 Dissolve Oxygen Consumption

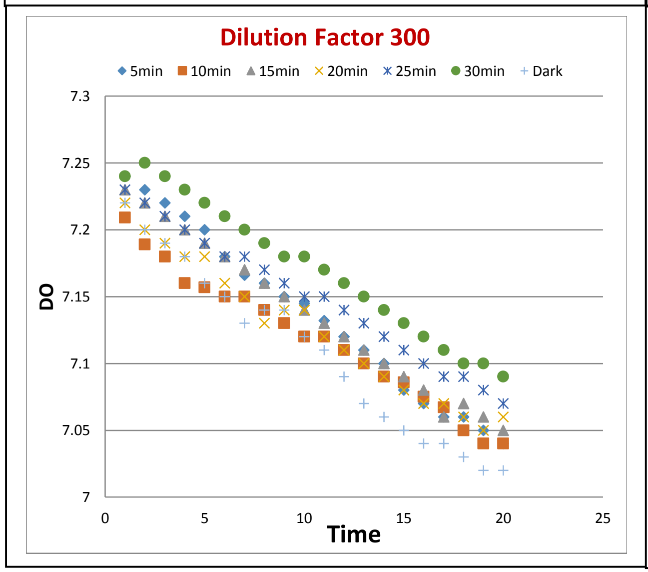
Oxygen Consumption of oxygen by copepods in control and dark control was measured using an NADH calibrated Polari graphic oxygen electrode system (Robinson and Cooper 1970, Reed 1972) and continuously recorded manually after every one minute for a total of 20 min.

In general, DO decreases with increase in exposure time as a function of measuring time. The oil samples that are subjected to sunlight for longer period of time shows less decrease in DO compared to dark control which may be attributed to the sessility of copepods.

Figure 4. 9: Following are shown as a function of different dilution factors


Dilution Factor 100
5min
10min
15mn
20min
25min
30min
Dark
DO
7.3
7.25
7.2
7.15
7.1
7.05
7
0
5
10
15
20
25
Time


Dilution Factor 200
5min
10min
15min
20min
25min
30min
Dark
DO
7.3
7.25
7.2
7.15
7.1
7.05
7
6.95
0
5
10
15
20
25
Time


Dilution Factor 300
5min
10min
15min
20min
25min
30min
Dark
DO
7.3
7.25
7.2
7.15
7.1
7.05
7
0
5
10
15
20
25
Time

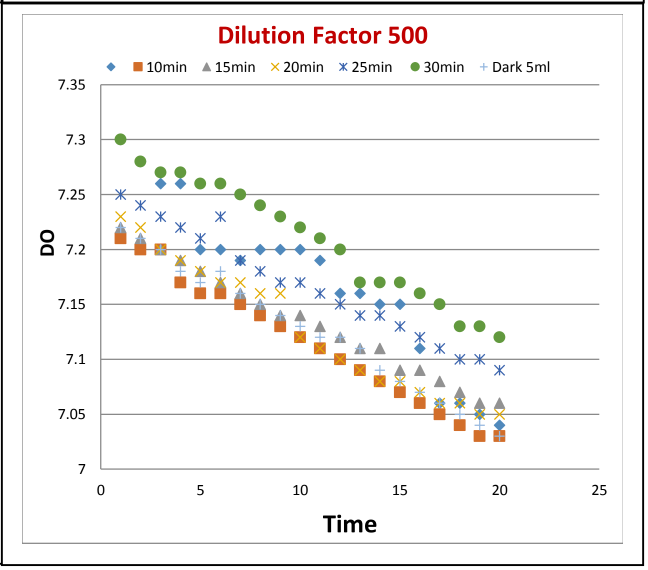
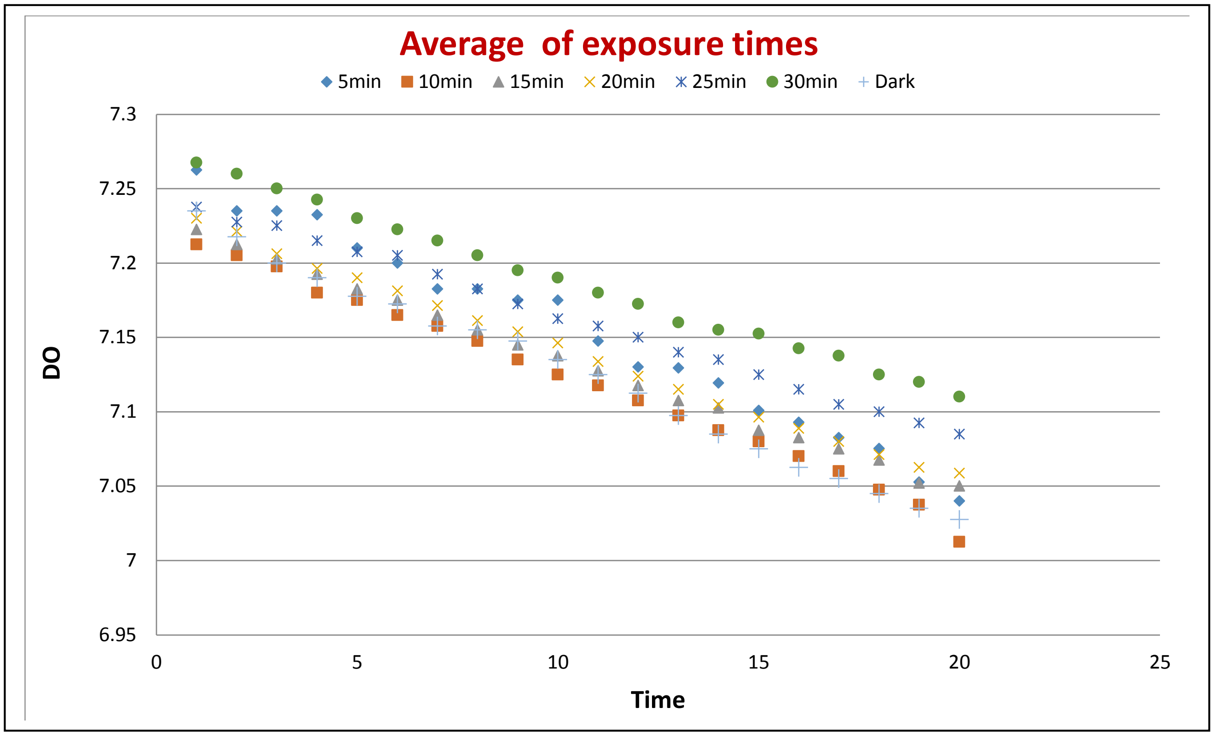

Dilution Factor 500
10min
15min
20min
25min
30min
Dark 5ml
DO
7.35
7.3
7.25
7.2
7.15
7.1
7.05
7
0
5
10
15
20
25
Time

Figure 4. 10: Average of Dissolve Oxygen of Dilutions



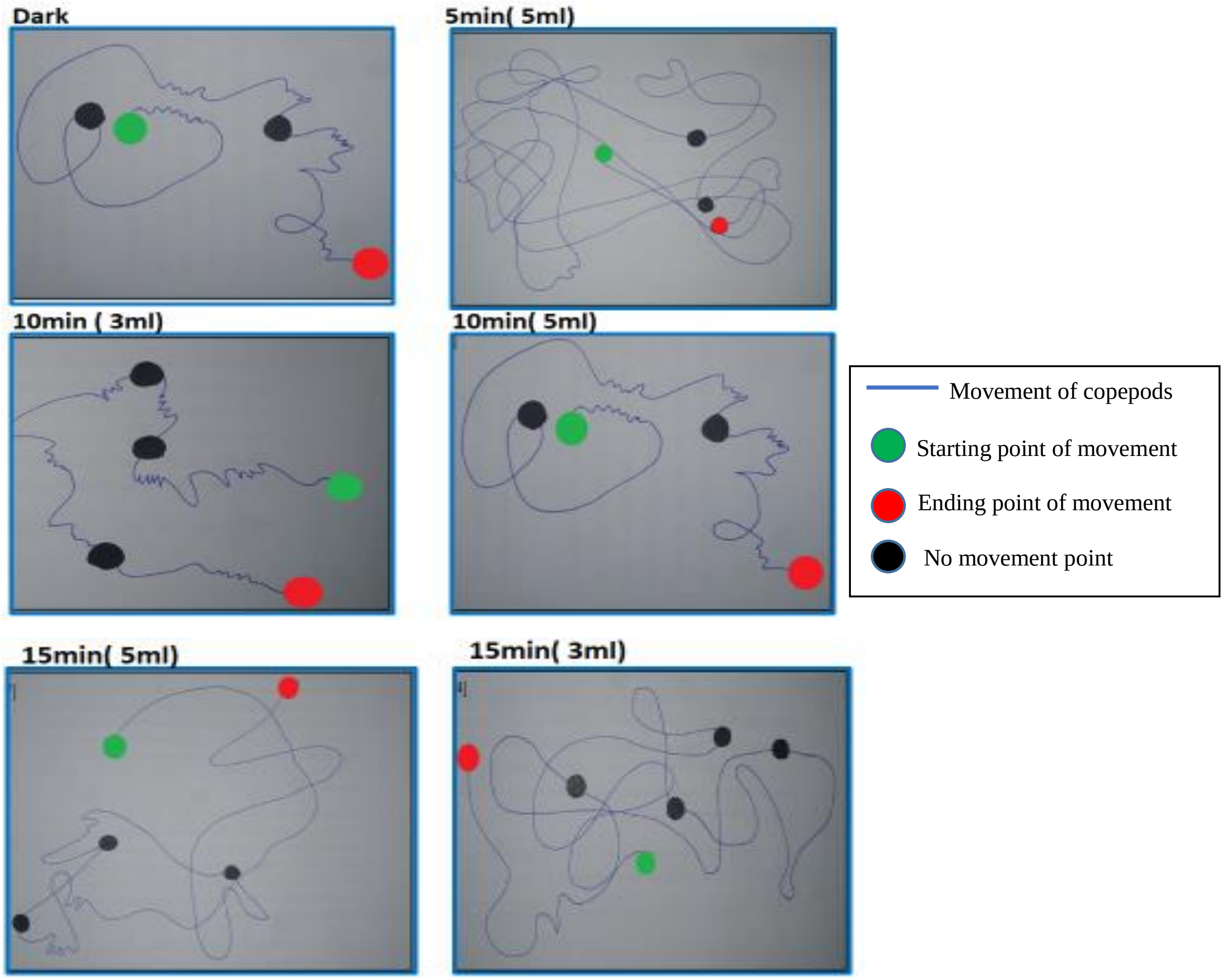
This is the summary of all the exposure times averaged for different dilutions which shows with increase in oil concentration and exposure to simulated sunlight caused decreased in the availability of dissolved oxygen which decreased the survival ship of test organisms.

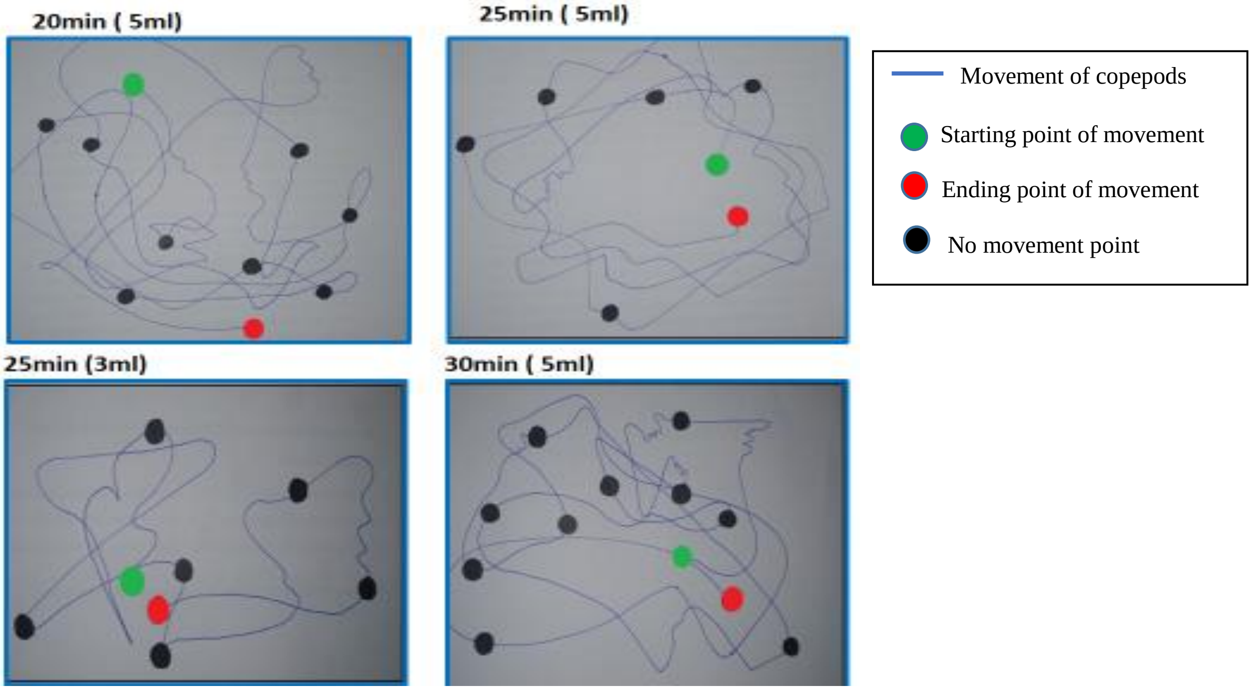
4.4. 2 Swimming Behavior

Swimming behavior, pattern of movement and orientation to patches of copepods were recorded using microscope. Swimming speed of animals were determined in a slide having seawater and observed under microscope (0.14 W em-“, wavelength Z- 625 nm) with a 35-mm SLR camera and high- speed black and white film (ASA 1600).

A total of six samples were taken for 5, 10, 15, 20, 25, and 30 min exposure to sunlight. Each sample was then made into four dilutions at which no lethal toxicity was noted then five males and five females Diacyclops were added to them and left for 24 hours. After

24 hours, each Diacyclops was drawn out and put into fresh SW. The swimming behavior for each Diacyclops was recorded for 10 min. A blank and a dark control for each experimental group are also analyzed, while their swimming behavior recorded under normal condition. In blank control, the Diacyclops have a slow, steady motion accomplished using mouthparts and succession of jumping to and fro, continuous and intermittent swimming. But in exposed sunlit samples, Diacyclops were increasingly restless and hyperactive, and were observed to have a loss of balance and equilibrium, irregular and iritic swimming, and decolourization of skin.





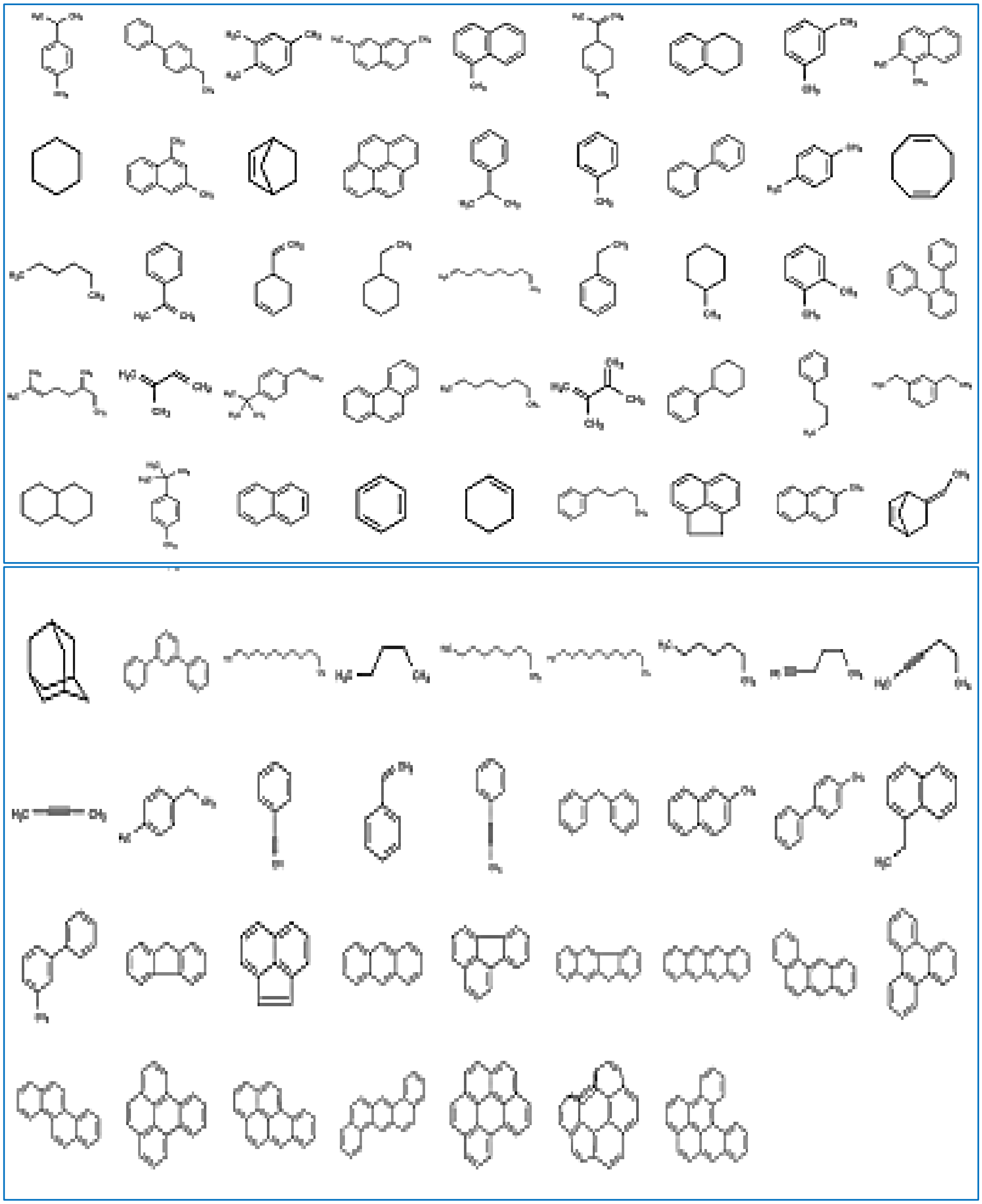
The above graphs shows increase in sessility of Diacyclops with increase in sunlit exposure time compare to dark control

4. 5 Passive samplers as alternative to animal

In this research, four phases of passive samplers were widely used i.e. PDMS (Polydimethylsiloxane), POM (Polyoxymethylene), PA (Polyacrylate) and Hexadecane. These samplers were compared with the fish Fatehead minnow to solve the hypothesis that the uptake by passive samplers is same as that by the fish.

The chemical set used for the evaluation of this hypothesis includes the following chemicals.

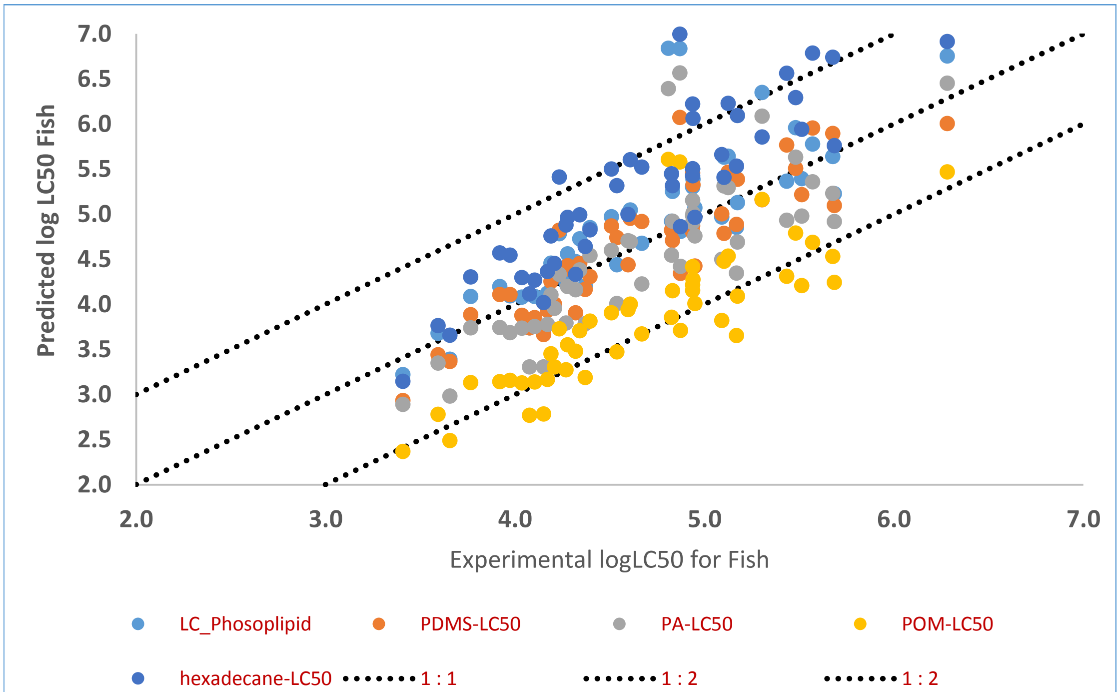
Figure 4. 11: Chemical set for petroleum hydrocarbons



4.5. 1 Results for Petroleum Hydrocarbons

For petroleum hydrocarbons, the model prediction exhibited a root – mean- square error (rmse) of 0.30 log unit when compared to experimental values of (n = 46) median lethal concentration as reported in literature. Whereas, previous model of critical membrane concentration (CMS) reported in literature returned higher rmse value of 0.35 log unit when compared to the same experimental data base (n = 46). The experimental data used for this evaluation was chemically diverse, and represented typical petroleum hydrocarbons including acyclic, aliphatic, cyclic aliphatic and cyclic aromatic hydrocarbons.

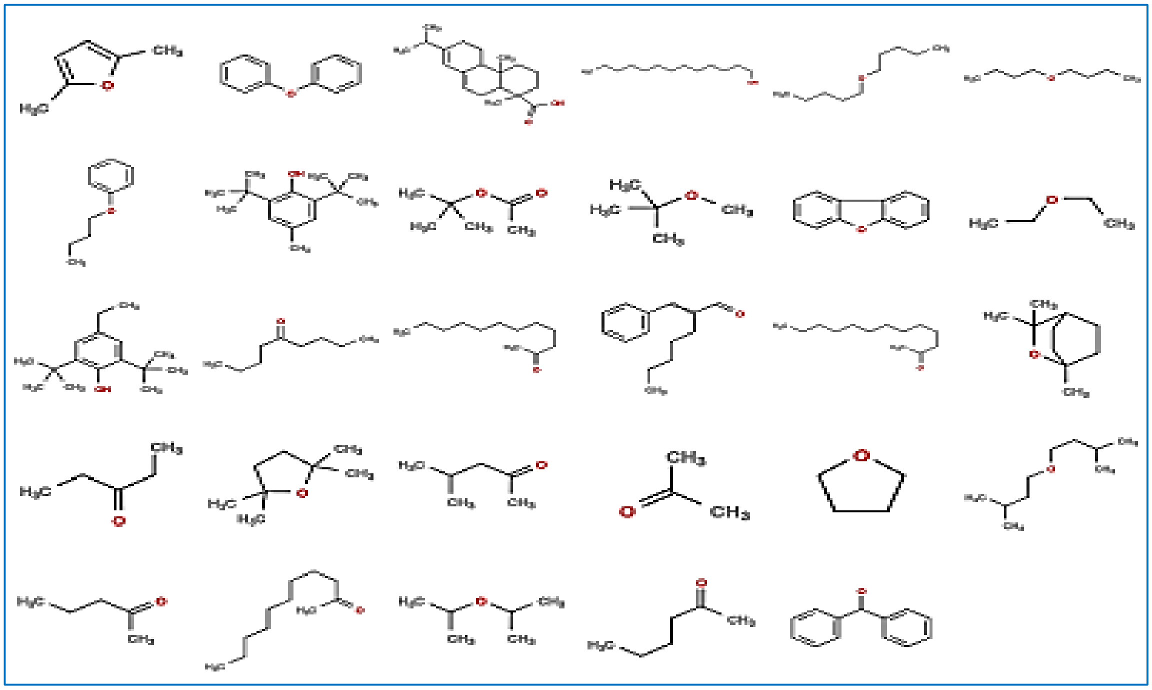
Figure 4. 12: Petroleum Hydrocarbons



Column1	Phospholipid	PDMS	PA	POM	Hexadecane
RMSE	0.35	0.3	0.4	0.85	0.66
N	46	46	46	46	46

4.5. 2 For Oxygenated Hydrocarbons

Figure 4. 13: For OxHCs, thirty chemicals were taken, the structure of which are shown below.



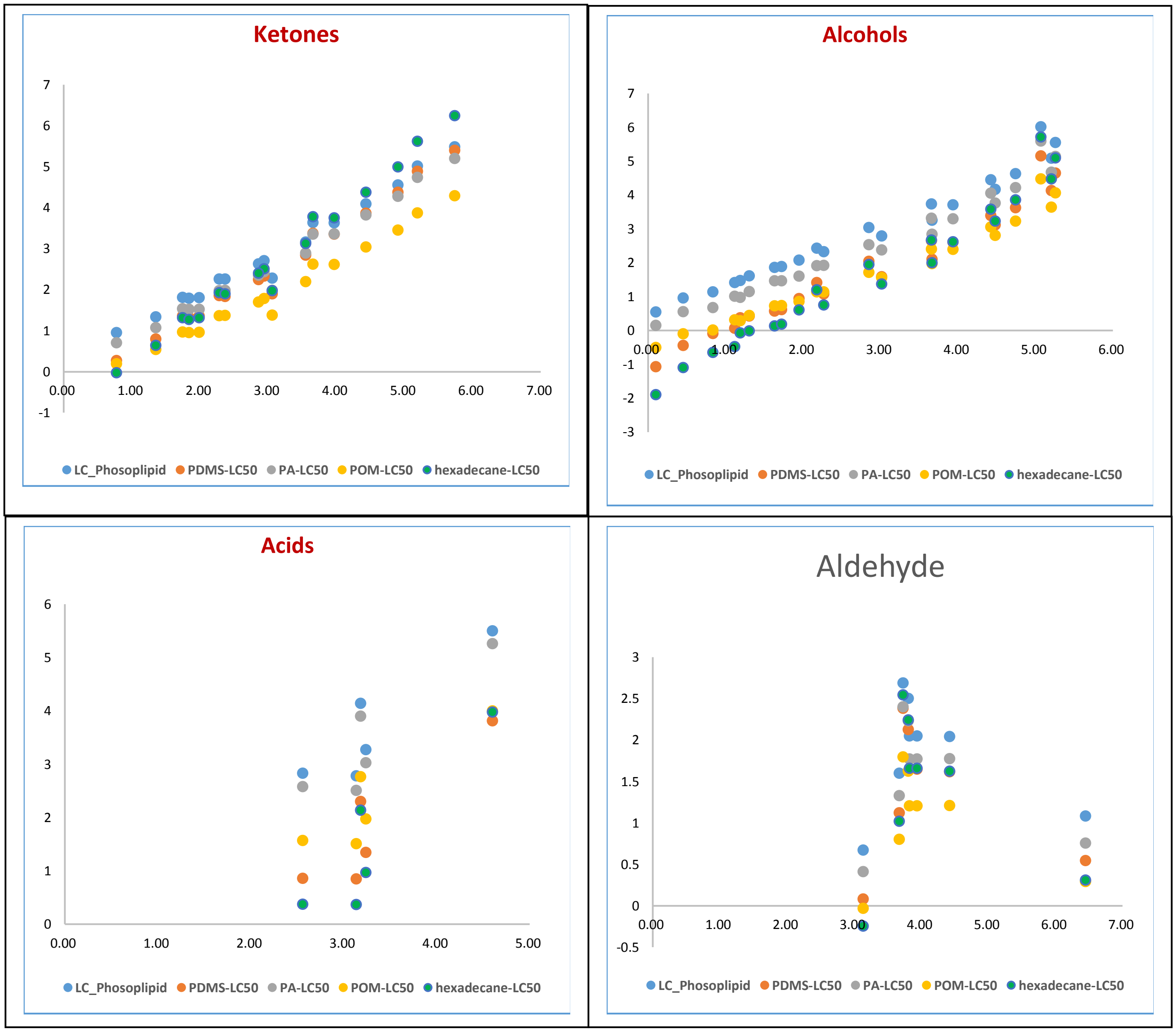
4.5.2. 1 Results for OxHCs

The research model is then evaluated for oxygenated hydrocarbons (OxHCs) transformation products which are reported to form in the weathered oil samples due to photo-oxidation and bio-oxidation. For chemical families that were detected in oil samples such as ketones (16), aldehydes (7), alcohols (n=22) and acids (n=5). They exhibited a root mean square error of Ketones 0.52, aldehydes 1.2, alcohols 1.1, and acids 1.5 respectively.

Higher the (rmse) value for OxHCs indicates that these chemicals follow toxic mode of action other than narcosis.

Similarly, the research model performed well for ketones and alcohols which follow base line mode of action while acids and aldehydes followed specific mode of action.

Figure 4. 14: Oxygenated Hydrocarbons



Column1	Phospholipid	Ketones	Alcohols	Acids	Aldehydes
RMSE	0.35	0.52	1.1	1.5	1.2
N	46	16	22	5	7

CONCLUSION & RECOMMENDATION

5.1 Conclusions

Photo generated transformation products of spilled crude oil in marine environment poses many adverse effects on aquatic life. The toxicity due to transformation products was experimentally determined using copepods as test organisms, and this was compared to the native hydrocarbons parent compounds. The effect of light jet oil on copepods have not been investigated in detail so far.

This study provided insight into the effect of light jet oil on copepods (*Diacyclop Bicuspidatus odessanus)* and use of passive sampling phase PDMS as an alternative to animals for toxicity assays

Finding of the present study are mentioned below

- Sunlight changes the chemical composition of oil
- Many OxHCs detected in sunlit oil samples
- Sunlit samples shows high lethal toxicity towards copepods
- Sunlight enhances the sub-lethal toxicity of oil samples
- 10% on average toxicity increases compare to dark in lethal
- 60%an average toxicity increase compare to dark in sub-lethal
- Passive samplers capture the baseline toxicity of HCs
- Passive samplers can discriminate the mode of toxic actions
- Acids and aldehydes products of the petroleum HCs do not follow baseline mode of action
- Ketones and alcohols followed toxic mode of action
- Model for HCs toxicity predict RMSE value of 0.3log unit compare to previous model unit when compared to the previous model which returned higher RMSE value (0.35 log unit) for same experimental dataset.
- Model for oxygenated hydrocarbons (OxHCs) show higher RMSE values indicating these chemicals have higher toxic mode of action than narcosis

5.2 Future Recommendations

In current study, toxicity due to transformation products of light jet oil was experimentally determined using copepods as test organisms. On the other hand transformation products showed adverse impact on lethal and sun-lethal toxicity endpoints. However there is a research gap in this field .Following are the recommendations for the work to be done in future,

- Oil samples are too complex for characterization so use GC×GC-MS for chemical characterization of oil
- In my research I look at lethal and sub lethal effects other chronic exposure end points such as reactive toxicities of OXHCs to DNA and Protein should investigated

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