Quantitative determination of BTEX in groundwater using dispersive liquid-liquid microextraction technique and GC-FID.

Doreen C Gwanyuki

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Wits University

DECLARATION

I declare that this research is my own, unaided work. It is being submitted for the Degree of Master of Science Coursework and Research at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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(Signature of candidate)

___30_____day of ____October ________20_22_______________at_____________

ABSTRACT

Dispersive liquid-liquid microextraction (DLLME) method was developed and validated for the extraction and analysis of aromatic hydrocarbons BTEX (benzene, toluene, ethylbenzene, xylene) in water samples. DLLME is a miniaturized version of liquid-liquid extraction (LLE) is a fast, efficient, and inexpensive microextraction method for pre-concentrating and extracting hydrocarbons.

In this study the DLLME technique was used for the determination of BTEX in water samples. BTEX normally exists in trace amounts in environmental samples thus sample preparation plays a vital role. Sample preparation played a significant role in extracting the analytes, pre-concentration of the sample and removal of interferences. This is important because GC-FID is a highly sensitive method and uses small volumes in the analysis. Sample preparation techniques such as liquid-liquid extraction are tedious, time-consuming, and insensitive, to counter these disadvantages and using large sample amounts of highly toxic hydrocarbons microextraction methods known as DLLME was used. The importance of sample preparation is frequently ignored but it is a significant step in the analytical method because accuracy and precision depend largely on this step.

Data obtained from this study compared with other microextraction methods from literature show that the extraction method demonstrated good precision, linearity, detection limit and enrichment factor for the extraction of the analysis.

Extraction parameters such as the type of extracting solvent, extracting solvent volume, type of dispersion solvent, and the volume of the dispersion volume were optimized. Under optimum conditions, the detection limits for DLLME ranged from 1.8 to 2.6 μg. L^{-1}, repeatability from 2 to 4% and the extraction efficiency ranged from 60.1 to 95%. The applicability of the DLLME method was studied by gas chromatography with flame ionization detection system. The results demonstrated that the environmental water samples contained trace quantities of BTEX compounds, 2.7, 1.9, 0.3 and 0.4 μg. L^{-1}, respectively.

DEDICATION

In memory of my father

Enock Muvirimi Zenda

1928-2004

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I would like to thank God for the energy, power, wisdom and understanding to be able to finish this research. My soul praises him for his faithfulness that endures forever.

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Special thanks to my children Shalom, Tawananyasha, my husband Philip for their patience during my studies. This should be an inspiration to you my children that you can achieve anything that you purpose in your heart and that the sky is only the beginning.

This work is dedicated to my mother and late father for their inspiration. They motivated me from a young age to advance my studies. My mother would always say, if I was young, I would go far with my studies.

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ABBREVIATIONS AND ACRONYMS

BTEX	Benzene, Toluene, Ethylbenzene, Xylene
DPH	Dissolved Phase Hydrocarbons
DO	Dissolved Oxygen
DLLME	Dispersive liquid liquid extraction
GC-FID	Gas chromatography flame ionization
GC-MS	Gas chromatography Mass spectrometer
HF-LPME	Hollow fiber liquid phase microextraction
LLE	Liquid liquid extraction
PHC	Petroleum hydrocarbon
NAPL	Non-aqueous phase liquid
LNAPL	Light non-aqueous phase liquid
MTBE	Methyl tert-butyl ether ($CH_3COCHCH_3$)
NSZD	Natural source of zone depletion
PDH	Dissolved phase hydrocarbons
PVH	Volatile Phase Hydrocarbons (PVH)
SPE	Solid phase extraction
TEAP	Terminal electron acceptor processes
TPH	Total Petroleum Hydrocarbons
VOC	Volatile organic compounds
WCOT	Wall- coated open-tubular

CHAPTER 1: INTRODUCTION

This introductory chapter gives an overview of the project, objectives and motivations as well as the key questions.

1. INTRODUCTION

Petroleum refers to a very complex combination of a variety of hydrocarbons of low and high molecular weight (Abha S and Singh C.W, 2012). Petroleum known as crude oil is refined into various fractions like petrol, naphtha, light oil, diesel, kerosene, asphaltenes, and lube oil waxes. The fractions distilled at atmospheric pressure are subdivided into two i.e., light fractions also known as lights ends and heavy fractions also known as heavy ends. The light end fractions have low molecular weight saturated hydrocarbons, unsaturated hydrocarbons, naphthenes, and low proportion of aromatic compounds; whereas the heavier end fractions consist of high molecular weight aliphatic, high molecular weight aromatic and organometallic compounds (Abha S and Singh C.W, 2012). Fig 1.1 shows typical structures of aliphatic petroleum hydrocarbons. Fig 1.2 illustrates the structures of aromatic hydrocarbons.

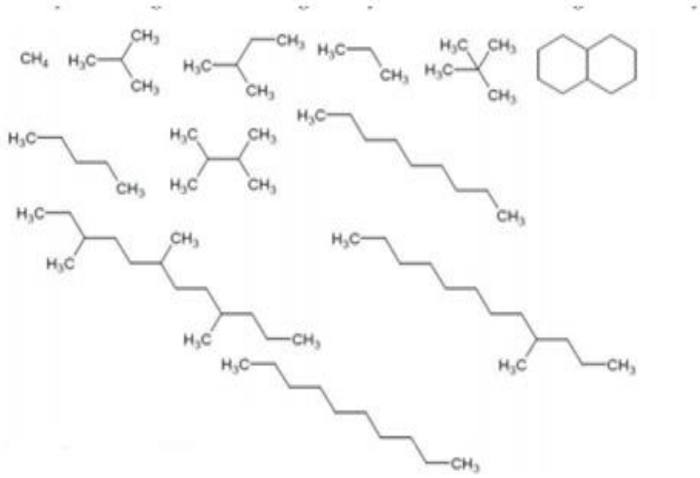


Fig 1.1 Typical saturated linear and branched chain hydrocarbons (Abha S and Singh C.W, 2012).

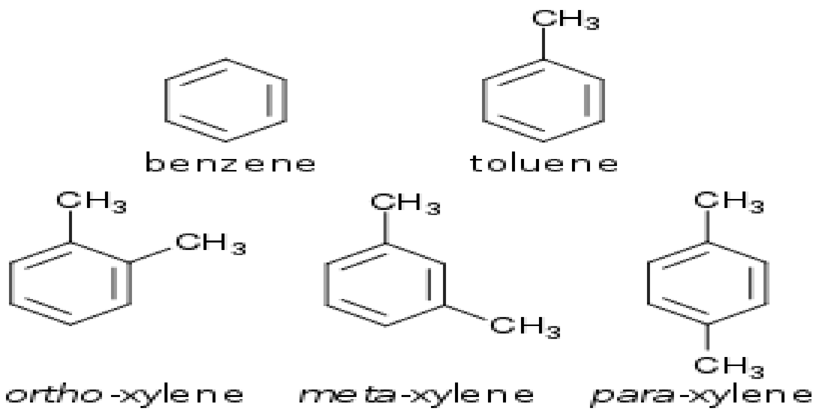


Figure 1.2 Structural formulae for benzene, toluene, meta, ortho, para xylene (Fayemiwo O.M et al, 2017).

Globally, environmentalists and scientists face the problem of overcoming the detrimental effects of contamination of soil, air, and water due to the increasing release of hydrocarbons and toxic substances (Ite A.E, et al; 2013). Pollution of the environment with petroleum(oil) and petrochemical derivatives has been identified as a substantial problem (Hsia KF et al., 2021).

Most oil components are harmful to wildlife and humans in general, as it is easy to add to the food chain. The oil components are consumed by bacteria, plankton and bottom-fed creatures which will be eaten by fish and in turn big fish thus affecting the food chain. This has provoked interest in investigating the fate, distribution and behavior of petroleum and its products in the environment (Fayemiwo OM et al., 2017). Oil spills in the environment cause long-term damage to aquatic and soil ecosystems, human health, and natural resources.

Failures of petroleum supply pipes and tanks of products at service stations occur frequently in many countries worldwide. (Fayemiwo OM et al., 2017). These failures have resulted in extensive groundwater contamination in areas where they have occurred. Rupture of underground pipes corroded underground tanks and sabotage of underground pipes have caused significant hydrocarbon impact of the subsurface (Hsia KF et al., 2021).

Oil spills are often associated with marine-related tankers or oil rigs accidents. In contrast, oil spills on the land tend to be overlooked by everyone except, environmentalists and yet onshore oil spills result in the pollution of the soil and water supplies. Typical sources of land oil spills include accidents as well as oil from vehicles on the road. There are so many contaminants in each product so that it becomes practically challenging to quantify each of them separately.

Spillages and leakages of petroleum products cause near-surface soil and water contamination and have the potential to contaminate portable water resources and the environment. Unconfined aquifer systems are particularly at risk (Fayemiwo OM, et.al; 2017). Quantification of oil leak and its derivatives is critical to interpret the behavior of oil and its lasting effects on the surroundings and to select the suitable remediation methods. Mono-aromatic hydrocarbons such as benzene, toluene, xylene and ethylbenzene (BTEX) are present in the environment typically found as volatile organic compounds (VOCs). BTEX are present in petroleum products and are released to the environment in the form of liquid spills or vapor losses. BTEX are common pollutants that are found in groundwater plumes and in some water resources due to disposal of contaminated industrial effluent and accidental oil spills or pipe leaks. (Fayemiwo OM, et.al; 2017). The impending danger which petroleum products pose to humankind and the surroundings make BTEX characterization and testing of the biotransformation and biodegradation processes of petroleum products in contaminated soil important. These characteristics enable the development of remediation techniques to clean the soils to levels that allow it to be safely disposed of or reused.

The behavior of petroleum contaminants and movement in the subsurface are important for the study because they affect contaminant levels in the environment when released to the environment. Petroleum contaminants degrade over time and distance (Duan X, Li V; 2017).

Pollutants from petroleum products can behave in various ways when exposed to the environment. When spilled in the environment petroleum products either sink to the subsurface or evaporate to the atmosphere (Barberes GA et al.,2017). Factors influencing the spread of contamination in the environment. Petroleum products include the solubility of contaminants as well as their ability to break down or bind to soil particles (Duan X, Li V; 2017).

Groundwater contamination by hazardous chemicals frequently involves incidental releases of petroleum hydrocarbon (PHC) mixtures, such as diesel fuel. A series of complex processes lead to mobilization of PHC compounds in the subsurface.

As a result hazardous compounds, such as Benzene, Toluene, Ethylbenzene, Xylene (BTEX) and low molecular polycyclic aromatic hydrocarbons dissolve into groundwater and may pose the danger described above. These compounds are degradable by microorganisms under various environmental conditions.

1.1 MAIN OBJECTIVE

The research aim is to determine the quantity of BTEX in the groundwater using the dispersive liquid-liquid micro-extraction technique and GC-FID.

1.2 SPECIFIC OBJECTIVES

a. To determine the quantity of BTEX in the groundwater using dispersive liquid-liquid microextraction technique and GC-FID.
b. To determine the difference between mobility or transport of dense hydrocarbons such as diesel and light hydrocarbons such as petrol.
c. To investigate the process of biodegradation or fragmentation and fragmentation patterns of petroleum hydrocarbons in soil and water

1.3 RESEARCH QUESTIONS

How effective is the dispersive liquid-liquid microextraction technique in extracting the BTEX for analysis using GC-FID?

1.4 MOTIVATION

In South Africa and the world in general groundwater pollution by petroleum hydrocarbons is becoming a challenge and therefore there is need for studies to be conducted to understand the impact of these pollutions on the environment and its ecosystem. Many oil industries worldwide are struggling with the problems of oil spillages and leaks due to past mistakes in installing the proper infrastructure which prevented their products from entering the environment.

Generally, ingesting petroleum products or hydrocarbons such as gasoline and kerosene cause irritation of the throat and stomach, central nervous system depression, difficulty in breathing, and pneumonia. Long-term impact of exposure to petroleum products could lead to the development of cancer as some of these products are known to be carcinogenic. This prompts studies to be done in this area to reduce the impact on human beings and animals.

CHAPTER 2: LITERATURE REVIEW

This chapter presents a concise review of the literature relating to petroleum products and their constituents with emphasis on their mobility and biodegradation. Emphasis was made on the following areas: - formation of light non-aqueous phase liquid plumes, mobility in porous media, migration in fractured rock and distribution thereof. Focus will also be on the fragmentation in water and soil including natural processes such as natural attenuation.

2.1 TRANSPORT OF HYDROCARBONS IN THE ENVIRONMENT

BTEX organic compounds have high molecular weight, high boiling points, high density are insoluble in water, levels recorded at up to 1000mg/L. This is much higher than the allowable maximum contaminant level of 0.005mg/L and a total of 10mg/L in all three forms. BTEX solutions are flammable, toxic, carcinogenic substances, the presence of excessive amounts in the surroundings may lead to an adverse impact on water quality and thus endanger public health and welfare.

Hydrocarbons may occur in four different phases, in liquid, solid-phase held onto solid particles, and in gaseous phase because of their insolubility, establishing an individual phase called the non-aqueous phase liquid (NAPL) (Johnson P C et al; 2006). Frequently hydrocarbons are grouped into light non-aqueous phase liquids (LNAPL) that are less dense than water. BTEX form part of the LNAPL group. Most hydrocarbons such as light gas and fuels are less dense than water. In the event of a spill, migration of the pollutant vertically occurs due to the capillary and gravitational force. The capillary force is also responsible for lateral migration (Maletić S. et al, 2013).

When there is an extreme soil pollution, three phases of pollution may be detected. i.e.

(i) Separate Hydrocarbons Phases – pollutant in a liquid form (detected in some cases identical to a floating layer on top of the groundwater or it can be stuck in vadose zone permeable space (retained or adsorbed by capillary forces).

(ii) Dissolved Phase Hydrocarbons (PDH) –from hydrocarbons soluble fractions.

(iii) Volatile Phase Hydrocarbons (PVH) – formed by oil volatile compounds. If surface spills occur, hydrocarbons begin a lateral migration and a downward movement. Immobile residual organic compounds will be encountered along contaminant migration paths.

The pollutant migration in soil is determined by many factors such as the source activities, the pollutant solution released into the environment, the pollutant characteristics (volatile and soluble compounds, viscosity) then the properties such as soil texture, its terrain slope, permeability, porosity, and soil water-holding capacity (Maletić S. et al, 2013).

LNAPLs comprise typically of a predominantly organic hydrocarbon chemical mixture with a variety of toxicological and physical-chemical properties which might influence their risks and environmental fate (CL:aire; 2011). Their transportation in the subsurface is complicated, being a (LNAPL-air-water) multi-phase flow problem and is characterized by a build-up of hydrophobic LNAPL which is buoyant in the interface of a water table that can potentially redistribute vertically or migrate laterally (potentially seeping to receptors on the surface water) due to human-induced or natural fluctuations of the water table.

Additional groundwater wells and resources risks may come from the widespread migration of a plume of dissolved-phase which can grow from the source of LNAPL in the subsurface. However, these can be alleviated by biodegradation or natural attenuation process. Risks to ground surface receptors, such as, inhabitants of buildings, may originate from LNAPL components that are volatile form vapor plumes in the subsurface (CL:aire, 2011).

NAPL which is in contact with groundwater, and, the ongoing dissolution, advective-dispersive transport leads to growing plumes of dissolved hydrocarbon constituents. The dissolved hydrocarbons total mass increases steadily as the rate at which the mass dissolving from the sources of NAPL exceeds the rate at which it is removed by biodegradation and sorption. For large longitudinal dispersivities, plume fronts might travel significantly faster than the average ground-water flow velocities (McMichael T; 2003).

2.1.1 TRANSPORT IN POROUS MEDIA

LNAPL migration soon after release comprises of vertical transport because of gravity, combined with migration laterally due to geologic heterogeneity (Zuo R, et.al; 2021). If small quantities of LNAPL are released the spread will end in a partially saturated zone as the LNAPL is immobilized in the pores.

In the case of large quantities being released with adequate volume to subdue the capacity of residual soil retention, the LNAPL migrations downwards will continue, headed for the water-table. Once the LNAPL reaches the zone of saturation, the downward migration continues until buoyancy and the growing water quantity inhibits the migration vertically.

LNAPL starts migrating laterally at the periphery of the capillary except if adequate LNAPL potential energy is available for the LNAPL to replace water and infiltrate beneath the water-table. Under these circumstances, downward LNAPL infiltration continues till the pressure head is stabilized by the capillary pressure and ascending forces of buoyancy that are collectively known as either water displacement pressure or light non-aqueous phase liquid pore entry pressure (). The resultant LNAPL plume will continue to spread laterally in the direction of the hydraulic gradient of the water table generating an LNAPL head gradient reacting to the opposing forces. The LNAPL passageway leaves a trail of residual LNAPL as detached tumors (known as ganglia) and droplets. The development of the natural source zone is influenced by the LNAPL lateral migration at the water table in which the LNAPL can drive the water from the natural source zone into the capillary periphery. Light non-aqueous phase liquid will mound in response to buoyancy resistance and pressures from pores. The lateral spread will take place in a reaction to the force of gravity for LNAPL formation increasing the lateral gradient; the resistive forces of vertical migration are higher than the resistance laterally. The growth of the LNAPL body will last until the force driving the release dissipates or is balanced by the development of capillary pressures (Zuo R,et al; 2021). Therefore, the LNAPL pore entry pressure or water displacement discontinues and is brought to a halt by capillary forces through ambient conditions of groundwater flow resulting in dispersed LNAPL ganglia. The LNAPL natural source zone is partly stable. Volatilisation and dissolution form an ongoing natural source zone development increased by the degradation of the local dissolved phase plume. The redistribution of LNAPL plume will occur due to fluctuations of the water table or even seepage of LNAPL to adjoining waterbody receptors.

2.1.2 NAPL DISTRIBUTION IN THE SUBSURFACE

Variation of LNAPL distribution in the subsurface depends on several wettability and capillary forces through the imbibition process. The movement of LNAPL is resisted by capillary forces. LNAPL and air generally favors large pores and water prefers smaller pores. Soils are generally wet and therefore LNAPL requires large capillary forces to reduce the capillary forces of water in the throats of small pores. In fine grain silts or clay, large capillary forces are increased by the wetting water being forced into the fine pores by the progressing non-wetting light NLAPL.

LNAPL develops a path preferentially of least resistance resulting in a heterogeneous irregular distribution of dislocated ganglia due to drainage of the LNAPL from pores (Oostrom et al 2006).

The size and type of LNAPL may also influence the distribution thereof. In cases of catastrophic releases because of tank failure, LNAPL elevation head will be large initially and declines gradually. However, when there is a continuous pipe leak the elevation head may grow constantly resulting in an LNAPL plume. In the case of a huge catastrophic release of LNAPL, the elevation head disintegrates rapidly, resulting in reduced infiltration below the water table with migration laterally driven by the difference in gradient resulting in disintegration and become self-limited.

A continuous release of LNAPL will eventually overcome capillary pressure and buoyancy at the water table and force the LNAPL below the water table and less lateral migration for an equal volume released (Sheng Y, et.al; 2018).

The viscosity of LNAPL also influences the length of time for stability. Low viscosity LNAPL e.g., petrol can migrate downward at an increased rate and achieve hydrostatic equilibrium within a range of few days or months. Increased LNAPL viscosity, for example crude oil may migrate at a lower rate through the subsurface and reach hydrostatic equilibrium after a few years.

(Praseeja A.V, Saijakumar N; 2021). The greater the difference between densities of water and LNAPL, the stronger the resistance, the less the vertical infiltration of the LNAPL and the greater the lateral migration.

2.1.3 LNAPL MOVEMENT WITH WATER TABLE FLUCTUATIONS

The fluctuation of the water table due to change in seasons or abstraction influences the emergence of LNAPL in the wells and aquifer (Sheng Y, et.al; 2018). Yearly water table variation ranges from 1m for gravels, sandstone, and porous sands, to more than 10 m in limestone and chalk aquifers where the porosity of the fracture signifies the capacity available for water storage. Observations from monitoring wells are as follows: -

- Monitoring wells may contain LNAPL which is not observable, although soil sampling reflects elevated LNAPL in the nearby formation.
- LNAPL thickness in monitoring wells for unconfined aquifers often reduce with the rise in water table and increase when the water table falls.
- In confined aquifers the LNAPL thickness in monitoring wells rise with the water-table and reduce when the water table falls.
- Unexpected disappearance and appearance of LNAPL in monitoring wells.
- If the water table falls below the preceding fluctuation range, LNAPL may disappear from the monitoring wells (Praseeja A.V, Saijakumar N; 2021).

The observed reduction and increase of LNAPL thickness in unconfined aquifer monitoring wells with the increase in the water table or vice versa are because of spreading and redistribution of mobile LNAPL in the plume. (Praseeja A.V, Saijakumar N; 2021). The kinetics of LNAPL illustrates the behaviour of petroleumproducts in the subsurface.

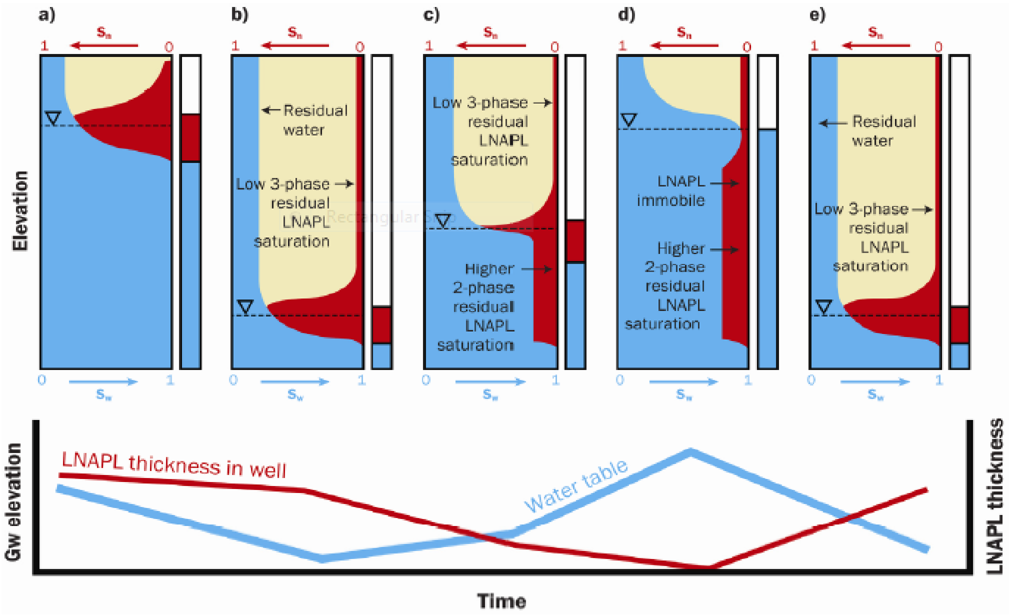


Fig 2.1 Observed variation of the LNAPL (CL:AIRE, 2014).

2.1.4 MIGRATION OF LNAPL IN FRACTURED ROCK

Fractures produce discontinuities in which LNAPL infiltrates and accumulate (Azimi R eta al., 2020). The type of rock porosity and permeability might or might not be significant. However, the rock fractured system therefore, exercises the dominant control. In a zone which is partly saturated, LNAPL migrates downwards through sub-vertical and vertical fractures due to gravity (*CL:AIRE, 2014*). The steeper the fracture dip, the bigger the effect of gravity, the lesser the LNAPL residual saturation. The flow of LNAPL increase with the decrease in fluid viscosity and as the fracture cavity increase. As the density of LNAPL-water mixture increase, vertical forces driving the LNAPL-water experience resistance from LNAPL buoyancy that stimulate lateral migration of the LNAPL at the water table through release and after water table fluctuations (*CL:AIRE, 2014*). Substantial lateral LNAPL spread may occur along the fracture dipping in the zone which is partially saturated. Fig 2, illustrates migration in fractured rock as described in (*CL:AIRE, 2014*)

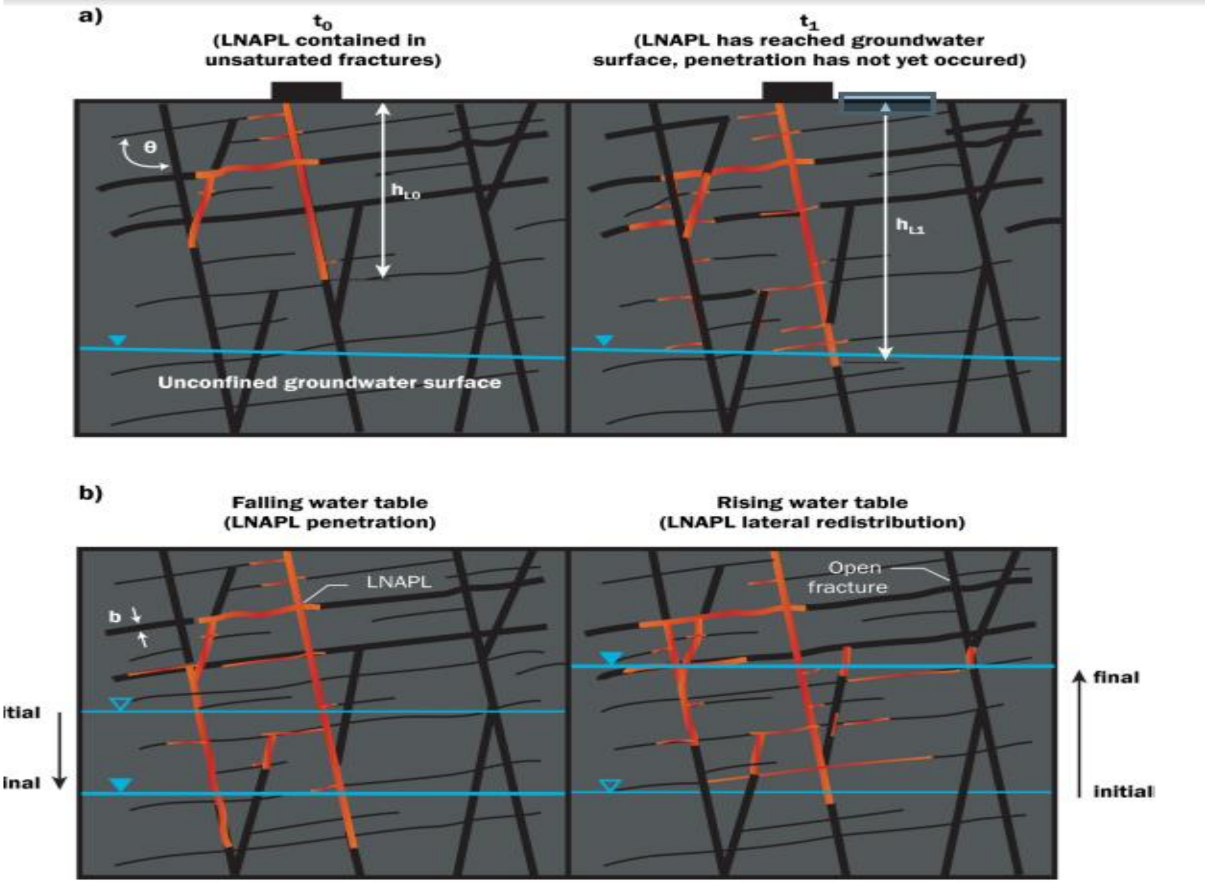


Fig 2.2 Migration in fractured rock (CL:AIRE, 2014).

2.2 FRAGMENTATION OF PETROLEUM HYDROCARBONS

Biodegradation is when microorganisms mineralize or transform organic pollutants through enzymatic or metabolic processes, that are incorporated into the cycles of natural biogeochemical. Hydrocarbon substances may be broken down through two biodegradation processes i.e. (a) aerobic, with oxygen, or (b) anaerobic, without oxygen. Anaerobic processes are completed by anaerobic microorganisms and this biodegradation pathway takes a long period of time. Anaerobic biodegradation processes have gained more attention due to the increase in data with regards to the rapid depletion of oxygen and information on conditions of contaminated site (Praseeja A.V, Saijakumar N, 2021).

Biodegradation of petroleum products anaerobically follows diverse biochemical pathways depending on the electron acceptor used by the microorganism. Petroleum pollutants have been proved to break down under several anaerobic conditions, i.e., sulphate, manganese , nitrate iron and methanogenic reductions, as well as ferric conditions. These metabolic pathways under anaerobic biodegradation are not yet fully understood. Most of the studies done associated with anaerobic mineralization of alkane hydrocarbons were conducted in laboratories with enrichment or pure cultures (Wang B, et.al; 2021). Thus, the importance of the results in the natural environment for example in sediments and soils that are contaminated is unknown and the data for the alkanes degradation anaerobically in the environmental has only been reported in few cases.

“Petroleum compounds contain a complex mixture of hydrocarbons; all the different components of petroleum do not biodegrade at the same rate. The rate at which microorganisms’ breakdown Petroleum hydrocarbons are dependent upon their organic structure and concentration (Kanwal M et al; 2022). Petroleum compounds can be classified into four components i.e., aromatics, saturates, asphaltene and resins. Of the varied fractions of petroleum, intermediate length (C_{10}-C_{25}) n-alkanes are the microorganisms preferred substrates and are often the most easily degradable, although compounds with a shorter chain are somewhat more toxic. Solid hydrophobic alkanes with a longer chain (C_{25}-C_{40}) subsequently are tough to degrade because of their poor bioavailability and water solubility. Cycloalkanes and branched chain alkanes are degraded more slowly compared to the normal alkanes. Highly condensed aromatic and cycloparaffinic structures, tars, bitumen, and asphaltic materials, have the highest boiling points and exhibit the greatest resistance to biodegradation. It has been suggested that the residual material from oil degradation is analogous to, and can even be regarded as, humic material (Kanwal M et al; 2022).

Biodegradability of petroleum hydrocarbon in soil is influenced by an array of factors, such as pH value, nutrients, oxygen, concentration, composition, and bioavailability of the contaminants and the soil's chemical and physical characteristics. Biodegradation is a major weathering process of crude oil and an important natural attenuation process.

Generally, petroleum hydrocarbons and other organic molecules with abundant carbon-hydrogen bonds are good food sources (electron donors) because they contain high-energy electrons. Soil and groundwater bacteria use a variety of natural electron acceptors in the degradation process. The use of these final electron acceptors is not arbitrary but is based on energy transfer efficiency and availability. The most common inorganic electron acceptor in ground water is dissolved oxygen (DO). Once DO has been depleted, bacteria will preferentially use the next most efficient electron acceptor—usually this is nitrate (NO_3^-) or insoluble manganese (Mn^{4+}). After NO_3^- and Mn^{4+} have been depleted, the bacteria will use ferric iron (Fe^{3+}), followed by sulphate (SO_4^{2-}), and carbon dioxide (CO_2), respectively. During the reduction of these electron acceptors CO_2, ammonia (NH_3), soluble manganese (Mn^{2+}) and iron (Fe^{2+}), sulphide (S^{2-}), and methane (CH_4) are produced (Kanwal M et al; 2022).

Bacteria responsible for biodegradation are categorized by their terminal electron acceptor processes (TEAP). Types of bacteria include aerobic bacteria, which use DO as their TEAP, nitrate-reducing bacteria, iron- and manganese-reducing bacteria, sulphur-reducing bacteria, and methanogenic bacteria. *Pseudomonas* bacteria are free-swimming aerobic bacteria known to degrade BTEX (Hsia KF et al., 2021).

Biodegradation rates for the various types of petroleum hydrocarbons depend on the TEAP occurring. The sequence of preferential electron acceptor processes has been shown to cause zones of different electron-accepting processes dominating in different redox zones in contaminant plumes (Kanwal M et al; 2022). Geochemical and microbiological data can be used to delineate the zones and to obtain information on possible degradation rates. Biodegradation rates of low to moderate weight aliphatic, alicyclic, and aromatic hydrocarbons can be high if ideal conditions are present. Resistance to biodegradation typically increases as the molecular weight of the hydrocarbon increases (Logeshwaran P et al; 2018).

At the Bemidji Minn India crude- oil spill site, microorganisms have degraded BTEX compounds in the ground water, and biodegradation has slowed the movement of BTEX compounds (Hsia KF et al., 2021). Many of the soluble hydrocarbons are degraded in an anoxic zone that has developed downgradient of the oil body, but benzene has been more recalcitrant under anoxic conditions and has migrated farther downgradient than other BTEX hydrocarbons (Eganhouse et al; 1996). Field studies at a crude oil spill site in India indicated that up to 75 percent of the hydrocarbons present could be biodegraded within a year if the proper geochemical and microbial consortia are present (Gogoi B.K et al., 2003). Multiple lines of evidence generally are needed to demonstrate biodegradation processes at contaminated sites (National Research Council, 1993; Wiedemeier T.H et al,.1995).

2.2.1 DISSOLUTION

NAPL in contact physically with underground water will solubilize and partition into the liquid aqueous phase. The solubility of the hydrocarbon is the equilibrium concentration of a substance in water at a specified temperature and pressure. The solubility therefore, denotes the concentrated compound in water.

The effective solubility equivalent to the maximum concentration which can be obtained in the explanation above is illustrated by the Raoult's law equation (i) (Derek et al, 2014)

$$I=X_iS_i \quad \text{…………………………} \quad (1)$$

I is the effective solubility of a compound in NAPL solution, X_i is the mole fraction of a compound i in NAPL solution, and S_i is the aqueous solubility of the pure-phase compound.

The LNAPL and constituents' effective solubility change with time since the soluble constituents preferentially dissolve in water resulting in changes in the mole fraction. The more soluble LNAPL dissolve quickly resulting in increased dissolution of aromatic hydrocarbons as the mole fraction increases. The changing composition of LNAPL results in time variant considerations in risk and remediation. In most petrol formulations, the light hydrocarbons and MTBE preferentially dissolve from the source of LNAPL at moderately high concentrations followed by BTEX dissolution. The hydrophobic non-polar hydrocarbons are significant, and these include straight and branched chain alkanes which are more persistent due to their sparing solubility. As a result, LNAPL hydrocarbons dissolve progressively leaving persistent and less soluble residue in the source. Although effective solubility estimates are best appropriate for mixtures where the organic components have similar structural sizes and polarity, the Raoult's law is a reasonable indicator of effective solubilities of mixtures of LNAPL. The hydrocarbon concentration ratios close to the source mirrors the effective solubility of constituents in the absence of processes such as biodegradation and sorption. Table 1.1 below illustrates the solubilities and partitioning coefficients of petroleum hydrocarbons in water.

Table 1.1: Solubilities and partition coefficients of petroleum hydrocarbons in water (Headley J.V et al, 2000).

Analyte	Solubility	Partition Coefficients
Benzene	1780*	2.37**
Toluene	515*	2.80**
Ethyl benzene	152*	2.92**
m, o Xylene	198*	2.98**
p-Xylene	175*	2.98**

2.2.2 VAPORISATION

Most light non-aqueous phase liquids have volatile organic compounds (VOCs) that are capable of vaporizing during and after the migration of LNAPL in the subsurface. This leads to the formation of a partially saturated zone of the vapor-phase plume (Molins S et al, 2010). The vapor phase plume may develop rapidly creating a source zone in different directions to the groundwater plume.

Impact on vapours may decrease in areas where migration of vapors occurs in the ground surface and vanish into the atmosphere. On the other hand, increased contamination by volatile organic compounds may occur through migration of vapor downwards (Rivett M.O et al, 2011).

LNAPL existing in the zone, which is partially saturated, enhanced by further coating due to the fluctuation of the water table, may volatilize into soil gas inside the partially saturated zone. This transfer of mass is dependent on the chemical vapor pressure. In a multi-component LNAPL individual component can be estimated by Raoult's Law.

$P_i=X_iP_i$ ………………………………………………………………………………………… (2)

Where X_i is the NAPL mole fraction of chemical I, and Pi is the pure phase vapor pressure.

As per effective solubility, preferential vaporisation of most volatile non-aqueous phase liquid components occurs. BTEX compounds in petroleum fuels vaporize as follows B>T>E >X. Vaporization is not very important for diesel and other heavy oils/fuels. The key parameters such as vapor pressure are sensitive to temperature fluctuations. This results in sensitivity to temperature for profiles in the shallow subsurface that may be above 40 ^{o}C in summer and winter in other countries (USEPA, 2013).

2.2.3 ADSORPTION

Most contaminants cannot enter the groundwater or migrate off-site, because they adsorb onto the soil particles (USEPA, 2013). The sediment particles and soil through which the dissolved contaminants and groundwater travel can sorb the molecules of the contaminants onto the particles of the surfaces. This holds bulk liquids in the openings between the particles, thus reducing or discontinuing the contaminants movement. This process can significantly reduce the probability of the contaminants reaching the location where directly affect human or environmental health (USEPA, 2013).

2.2.4 NATURAL ATTENUATION

The dissolved-phase plume composition that develops down the LNAPL gradient LNAPL source is closely related to the LNAPL composition and its developing vaporization and dissolution characteristics. The LNAPL source depletion will occur naturally through diverse processes alongside dissolution as it supports the development of dissolved phase plume in the groundwater. The processes accounting for the natural source zone depletion (NSZD) comprise of vaporization, dissolution, and biodegradation which is sustained by exchange of electron acceptor with water and gas in the saturated or unsaturated zone. These processes may improve mass transfer from the light non-aqueous phase liquid (CL:AIRE:2014).

Selective dissolution of the more volatile and soluble LNAPL constituents leads to dissolved plumes from fuel discharges that are dominated by benzene, toluene, ethylbenzene, and xylene (BTEX) components and highly soluble fuel oxygenates.

BTEX constituents are the key contaminants of concern in assessing risks modeled by petroleum products (Bowers R.L, Smith J.W.N, 2014). Complexity evolves over time when different LNAPL components dissolve, for example, in a setting with fractured chalk aquifers the petroleum spill scenario in which MTBE plume develop initially in the groundwater because of their low attenuation and high solubility. This is normally followed by an extensive benzene plume. The more biodegradable and less soluble aromatic hydrocarbons such as Toluene, Ethylbenzene and Xylene form dissolved phase plumes that are limited to the source area of LNAPL (Bowers R.L, Smith J.W.N, 2014). Although, dissolution controls the interval of dissolved phase plume inputs and the size, composition of the plume is primarily controlled by the natural attenuation in the aquifer.

Dispersion dilutes the concentration of the contaminants whilst sorption and biodegradation which remove the contamination and slows down the migration of the plume are important processes for attenuation (USEPA, 2004a).

2.2.5 REVIEW OF EXTRACTION TECHNIQUES

2.2.5.1 LIQUID-LIQUID EXTRACTION

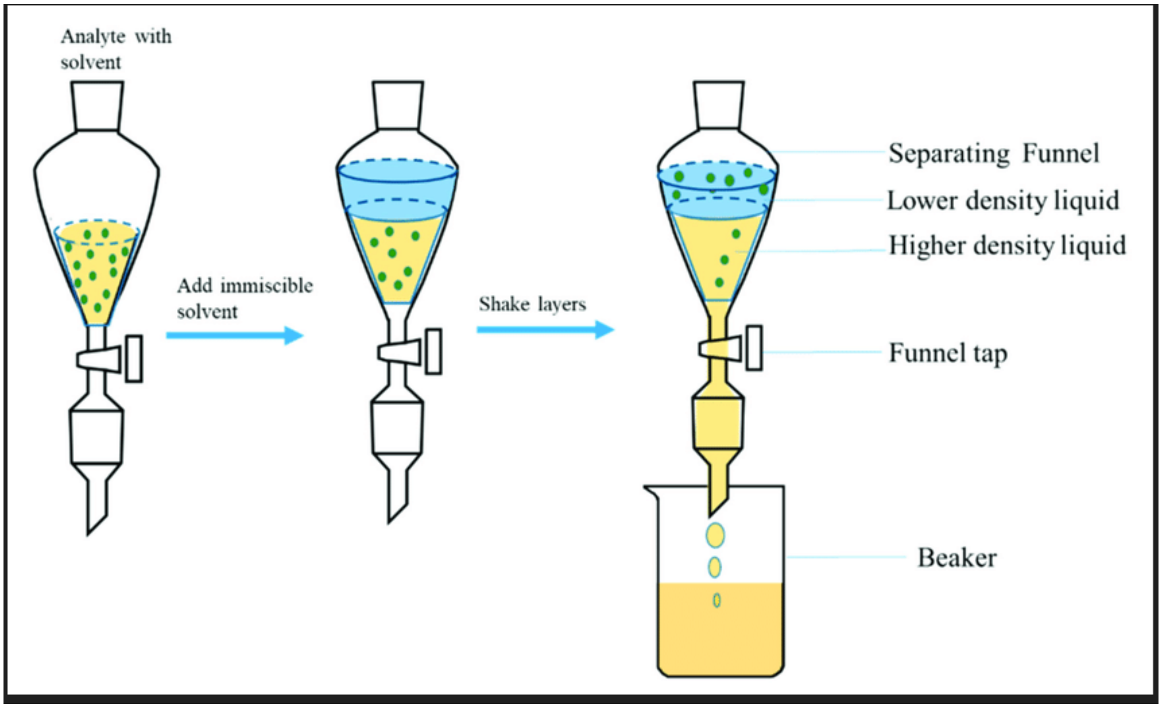


Fig 2.3 Liquid-liquid extraction technique diagram (Targuma S et.al; 2021)

Liquid-liquid extraction (LLE) is a technique used to separate two immiscible solvents that are components of a mixture based on the difference in their solubilities. The methodology utilizes the unequal distribution of components on two immiscible solvents. In liquid-liquid extraction, the aim is to separate the analyte of interest from its solvent by transferring it to another solvent. One of the liquid phases is a feed comprising of the carrier and solute. Another liquid phase (the extractant) is known as the solvent. The extractant must have a high selectivity, a good partition coefficient and easy separation from the products being extracted. The interfacial tension and viscosity are add-on important parameters. The extraction process is basically, the solute transfer between the feed and the solvent. The process starts by loading solvents into a column and then agitating with a mixer to allow the solvents to be thoroughly mixed. Once the agitation has been completed, the column is vented to relieve pressure. The mixed solvents can settle and then they are separated. The heavier solvent is then drained out of the column. The feed solution deprived of the solute becomes the raffinate and the solvent becomes the extract. Volatile solvents such as n-hexane, dichloromethane, benzene, ether, and ethyl acetate are normally used. n-Hexane is usually used for extraction of non-polar compounds from water e.g., aliphatic hydrocarbons. Benzene is used for aromatic compounds and ethyl acetate and ether are suitable for polar compounds. The extraction technique has a wide range of application in industrial processes such as, in the petroleum industry where there is simultaneous extraction of components with a wide difference in boiling points. However, LLE utilises large quantities of toxic and expensive organic solvents, is generally labour intensive and time consuming (Targum S et.al; 2021).

2.2.5.2 SOLID PHASE EXTRACTION

Solid phase extraction (SPE) is a separation technique that uses a liquid phase and a solid phase to separate analytes from solution. SPE is usually employed to clean up samples before chromatographic methods or other types of analytical techniques are used to quantify analytes in a sample.

The methodology involves loading a solution onto the solid phase then wash away the undesirable constituents. The desired analytes are then washed off into a collection tube with an alternative solvent. SPE uses the same kind of stationary phase which is used in the columns of liquid chromatography. The most used stationary phase is silica bonded to a defined stationary phase. The solid phase extraction stationary phase is held in a plastic or glass column above a glass wool. The column could have a glass wool above the stationary phase and could have a stopcock to manage the solvent flow through the glass or plastic column. SPE is centred on the analyte partitioning between the solid phase that is typically a sorbent contained in a column, a sample matrix in a liquid phase or a solvent containing analytes (Rocha FRP et.al; 2017). As a result of the distribution by adsorption or penetration of molecules on solid surface, an equilibrium is reached, and the analyte holds onto the solid surface in conformity with the distribution coefficient. Comprehending the mechanism of the SPE technique is key to the selection of the correct extraction sorbent. This is based on the interaction between the analyte of interest and the sorbent. The understanding of the mechanism also depends on the knowledge of the ionogenic, polarity and hydrophobic properties of the sorbent and the solute. The common retention mechanisms in solid phase extraction are centred on the non-polar interactions i.e., van der Waals forces, dipole-dipole forces, hydrogen bonding and ionic interactions. The retention from polar solutions of organic analytes on SPE materials is based to the attractive forces between the functional groups and the carbon-hydrogen bonds in analytes. Therefore, SPE uses the affinity of dissolved solutes in a mobile phase for stationary phase to separate a mixture into undesired and desired components. Sorbents offer unique properties which are useful in different types of extraction problems. Solid phase extraction method was used in the extraction of BTEX using water-soluble Beta-cyclodextrin polymer as a sorbent (Nojavan S, Yazdanpanah M; 2017)

2.2.5.3 DISPERSIVE LIQUID-LIQUID MICROEXTRACTION TECHNIQUES

Dispersive liquid-liquid microextraction is among the microextraction techniques that have grown popularity in analytical chemistry over the past few years. Assadi developed a new mic extraction technique known as DLLME (dispersive liquid-liquid microextraction technique) (Hasheminasab KS et al; 2013). This method is a miniaturized liquid-liquid extraction (LLE) which uses extraction solvents in microliters volumes. The extraction technique depends on the extension of contact surface between organic and aqueous phases. DLLME utilizes binary combination of two solvents i.e., extractant and dispersant for extracting and concentrating analytes from aqueous matrices (Diao CP et al; 2012). The role of the dispersion of the extractant solvent is to increase the surface area, thus increasing the extraction.

A suitable mixture of extraction solvent and disperser solvent which has a high miscibility in the organic solvent and aqueous phase is quickly injected into the aqueous mixture of the sample and it forms a cloudy solution due to the formation of droplets of the disperser and extracting solvent in the sample mixture.

The development of an emulsion permits instant analytes partitioning from aqueous phase to the extraction phase (a key advantage for the analytical technique). This is attained through the surface area which is large compared to LLE formed by the many microdroplets. The state of equilibrium is reached very quickly hence shortening the extraction time. The cloudy mixture is centrifuged causing the fine droplets to settle at the bottom of the test tube. This divides the cloudy solution into two-phased system enabling the easy extraction solvent recovery for analysis. The analytes of interest are extracted from the original sample and concentrated to a small volume and the analytes are determined in the settled phase using a specific analytical equipment such as GC-FID. DLLME is rapid, simple, inexpensive, sensitive and a green technique (Rahmani M. et al; 2015). DLLME uses dispersant solvents such as acetone, methanol, and acetonitrile (Diao CP et al; 2012). Water samples from Jajrooid river (Iran, Tehran) and Caspian Sea (Iran, Anzali) were collected and extracted using DLLME. BTEX compounds were extracted and analysed (Faraji H et al; 2013).

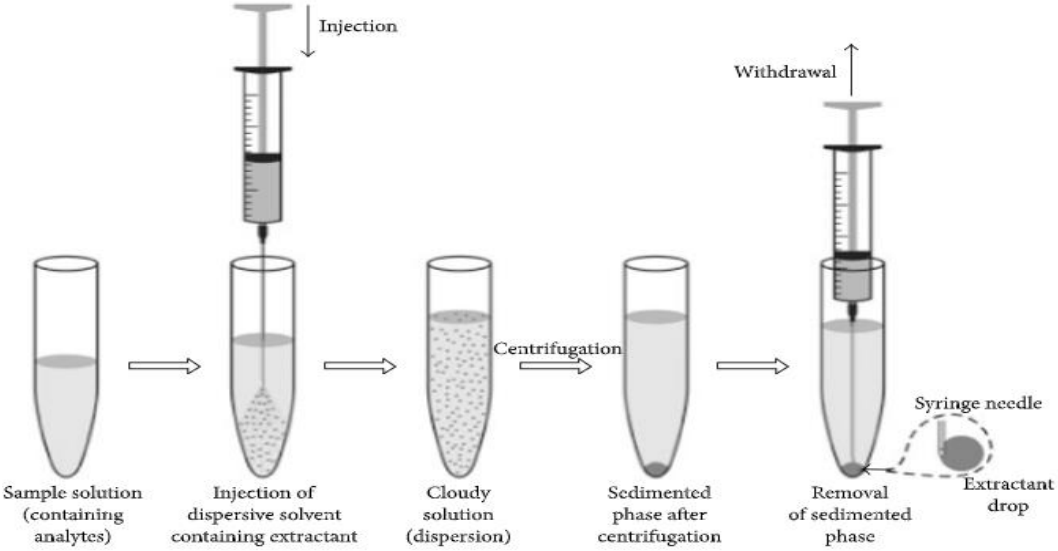


Fig 4.1: Schematic diagram of the DLLME apparatus (Quingley A, et al; 2016).

2.2.5.4 HOLLOW FIBER LIQUID PHASE MICROEXTRACTION

The hollow fiber liquid phase microextraction (HF-LPME) method is one of the microextraction technique that has been developed recently for the extraction of analytes from aqueous matrices. HF-LPME uses a hydrophobic membrane made from polypropylene which separates the organic phase and the sample. The quantities of organic solvents in the hollow fiber and inside the pores are much less than the traditional LLE technique. The extracting solvent wets the membrane pores, and the contact interface is at the membrane surface and the two phases never mix. HF-LPME can be accomplished in two or three phase modes. The method provides an excellent clean-up for the sample from contaminants. HF-LPME can be used in different configurations e.g., the fiber can be connected at one of the ends to the tip of microsyringe needle, the other end could be left suspended. The advantages of HF-LPME compared with other extraction techniques is that the hollow fiber protects the extraction solvent and allows the utilization of high stirring rates without droplet displacement (Sarafraz-Yazdi A et al.; 2010).

2.2.6 ANALYSIS OF HYDROCARBONS

2.2.6.1 GC-FID

The analysis of BTEX was done using the Gas Chromatography with flame ionization detector (GC-FID). The chromatographic method conventionally uses different detectors namely the electron capture detector, flame photometric detector, thermal conductivity detector and the flame ionization detector. The sample solution is injected into the gas chromatograph in minute quantities, and it enters the gas stream which carries the sample into a column. The different components are separated inside the column. The detector determines the quantity of the components that leave the column. To measure the quantity of an unknown sample, a sample with known concentration (standard sample) is injected into the gas chromatography. The standard sample peak retention time and area compared to the test sample to calculate the concentration. The flame ionization detector uses hydrogen gas to ionize sample constituents in the gas sample and quantify the resultant ion current.

GC-FID is generally good in the detection of the quantity of carbon in organic compounds.
After passing through the column, samples are heated in hot, hydrogen-air flame. Carbon ions are formed by combustion. The overall efficiency of the process is low (only 1 in 10^5 carbons ions produce an ion in the flame) the total amount of ions is directly proportional to the amount of carbon in the sample. Electrodes are used to measure the current from the ions. FID is destructive to the sample as the whole sample is pyrolyzed. Table 2.1 shows different instruments used in the analysis of total petroleum hydrocarbons (TPH) and benzene, toluene, ethylbenzene, and xylene (BTEX). The lowest quantity or concentration that can be detected by the GC-FID is between 0.1 to 0.2 μgL^{-1} and the minimum concentration detectable by a GC-MS is between 0.09-0.3 μgL^{-1}.

2.2.6.2 GC MS

Gas chromatography/ Mass spectrometry (GC-MS) is an analytical technique in which the separation technique of gas liquid chromatography is combined with the detection technique of mass spectrometry to identify components in a test sample. The MS identifies substances by the mass of the analyte molecule. It is a highly recommended technique for identification of environmental pollutants. Table 2.2. below shows when GC-MS instrument was used in literature.

CHAPTER 3: MATERIALS AND METHODS

This chapter is to expound on the details of the chemicals, equipment and procedure used in the research.

Optimization of several parameters to develop a method for analysing BTEX in water samples.

Extraction of BTEX using the dispersive liquid-liquid micro extraction technique (DLLME).

Analysis of BTEX in water samples using gas chromatographic technique.

3. CHEMICALS, STOCK SOLUTION AND INSTRUMENTATION

3.1 CHEMICAL AND REAGENTS

For the analysis of BTEX, analytical grade chemicals used were supplied by Merck (Damstadt, Germany). Dichloromethane, Chloroform, cyclohexane, and n-hexane were all supplied by Merck (Damstadt, Germany) as solvents for the traditional liquid-liquid extraction. For the Dispersive liquid-liquid microextraction technique, n-hexane, Cyclohexane, Chloroform, and tetrachloroethylene supplied by Merck (Damstadt, Germany) were used. Acetone, ethanol, acetonitrile, and methanol supplied by Merck (Damstadt, Germany) were used as the dispersive solvents for the DLLME method.

3.2 PREPARING STOCK SOLUTION

A stock solution consisting of 1 ml of 2000μgL^{-1} of BTEX from Sigma Aldrich (Steinhem, Germany) dissolved in 50 ml of methanol (analytical grade) from Merck (Darmstadt, Germany) was prepared. Working solutions were prepared daily from the stock solution by dissolving an appropriate amount of BTEX in methanol. The solution was kept at 4 ^{o}C.

3.3 INSTRUMENTATION

All samples were analysed on an Agilent Technologies 7890A GC-FID with CP-Sil 24CB (50% phenyl, 50% dimethylsiloxanne) capillary column, WCOT fused silica, 30 m x 0.32 mm ID with 0.25μm stationery film thickness.

A temperature program with an initial temperature of 28 ^{o}C for 2min was used. This was increased to 60 ^{o}C for 2min at 10 ^{o}C/min and it was further increased to 150 ^{o}C for 2min at 10 ^{o}C/min and finally increased to 200 ^{o}C at 10 ^{o}C/min for 2 min.

3.5 GENERAL EXTRACTION PROCEDURE

8 ml of water was added into a test tube followed by 15μL of extraction of solvent and 1 ml of disperser solvent. This was run in centrifuge for 10 min at 3000 rpm. Different extraction volumes were used 15, 20, 25 and 30 μL from a selection of extraction solvents i.e., cyclohexane, n-hexane, tetrachloroethylene, chloroform. The disperser solvents used were acetone, ethanol, methanol, and acetonitrile. Fig 4.1 shows the schematic diagram of the method of extraction of the analytes from the sample solution using the DLLME.

3.5.1 OPTIMISATION OF THE EXTRACTION

3.5.1.1 EFFECT OF VOLUME OF EXTRACTOR SOLVENT

The effect of volume on extractor solvent was investigated using 8 ml of distilled water spiked with 2 ml of 1 ppm BTEX standard. Extraction solvents used in this study included n-hexane, Cyclohexane, chloroform, and dichloromethane. Extractor solvent volumes were varied between 15, 20, 25 and 30 μL. 1 ml of acetone was added to the extractor solvent and stirred at 3000 rpm for 10 min. 2 μL was injected into the GC-FID for analysis.

3.5.1.2 EFFECT OF THE TYPE OF EXTRACTOR SOLVENT

The extractor solvent used for the study includes cyclohexane, chloroform, n-hexane, and dichloromethane. 8 ml of the distilled water was spiked with 2 ml of 1 ppm BTEX standard.

15 μL of the extractor solvent was added including 1 ml of acetone and this was rotated at 3000 rpm for 10 min. 2 μL was then injected into the GC-FID for analysis.

3.5.1.3 EFFECT OF TYPE OF DISPERSER SOLVENT

Dispersers such as acetone, ethanol and acetonitrile were used in this study. 8ml of distilled water spiked with 2 ml of 1 ppm BTEX standard was added to a vial.

n-Hexane was used as the extractor solvent and 1ml of disperser solvent was added and rotated at 3000 rpm for 10 min. 2μL was injected into the FID for analysis.

3.5.1.4 EFFECT OF VOLUME OF DISPERSIVE SOLVENT

The volume of the disperser solvent suitable for the study was investigated using 8ml of water spiked with 2 ml of 1 ppm BTEX standard. 15 μL of n-hexane extractor solvent was added to the solution and volumes ranging from 0.5 ml -2.00 ml were added. The solution was mixed at 3000 rpm for 10 min and 2 μL was injected into the GC-FID.

3.6 SAMPLING SITE

A service station in Rabie Ridge area was the sampling site used for this study. It is situated in Rabie Ridge adjacent to Freedom Drive around the Midrand area as shown in Figure 4.2 below. The service station in Rabie Ridge was chosen because there was a significant contamination of groundwater by petroleum products containing BTEX. The sampling results on site show that high values of BTEX in water.

Groundwater monitoring wells were drilled up the gradient and below the gradient to monitor the movement of the plume. Water samples were taken using a peristaltic pump. The non-aqueous liquid phase (NLAPL) was removed and then sampling was done. A peristaltic pump allows a representative sample to be withdrawn as it draws water from different points underground.

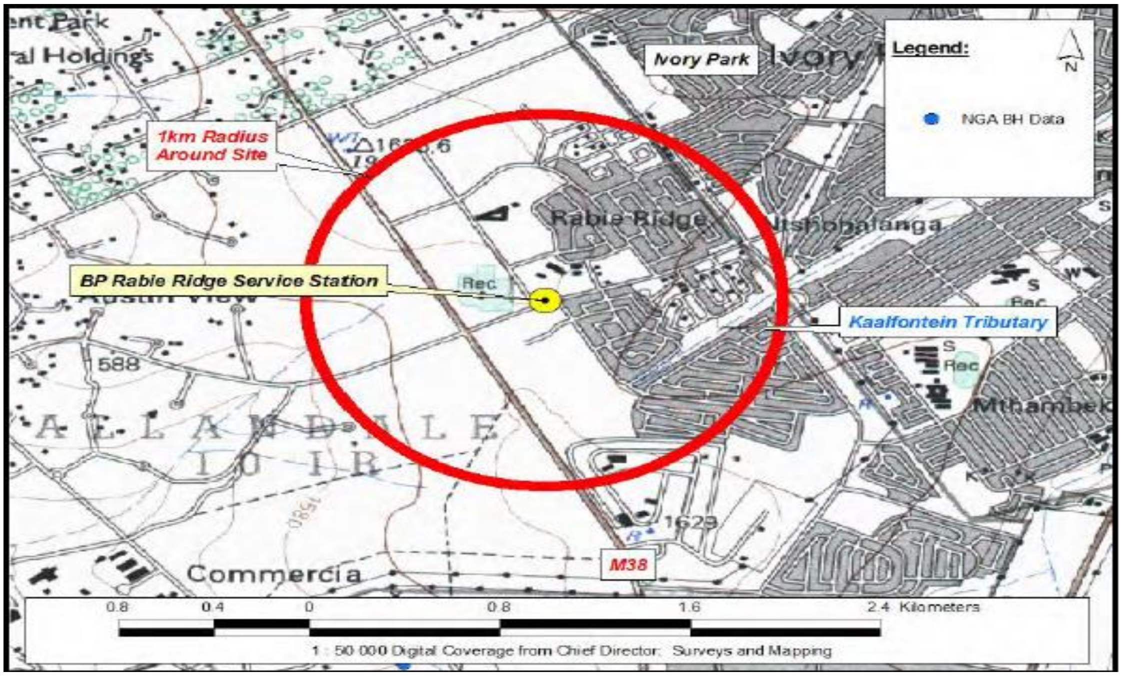


Fig 4.2 Sampling site map

3.7 PREPARATION OF WATER SAMPLES

Samples were collected from five ground water monitoring wells, in 500 ml brown bottles and were stored in a cooler box filled with ice. The sample bottles were cleaned in aqua regia and rinsed in distilled water. Columns They were stored at 4 ^{o}C until the day of analysis.

3.8 QUALITY ASSURANCE OF THE METHOD

3.8.1 LINEARITY

The linearity was assessed using calibration curves built by plotting average peak values versus the analyte's concentration. The values, show that the partition process is reproducible using these conditions, hence indicating that the method quantitative for these compounds.

3.8.2 SENSITIVE

The limit of detection based on signal to noise ratio of three (S/N=3).

3.8.3 PRECISION

The repeatability of the method was studied by analysing six replicate samples containing 1μgL^{-1} of each compound one after the other on the same day.

CHAPTER 4: RESULTS AND DISCUSSION

This chapter presents results and discussion for the experimental procedures outlined in chapter four. These results relate to the analysis of BTEX in samples extracted from groundwater contaminated with petroleum products.

RESULTS AND DISCUSSION

4.1 OPTIMISATION OF DLLME

4.1.1 EXTRACTION EFFICIENCY

The repeatability of the method was investigated by analysing 4 water samples. All the values were below 4% hence the precision of the method is acceptable. The robustness was evaluated on the extracting solvent volume using n-hexane because it was the parameter with the probability of variation. The robustness was performed on n-hexane volume. In the procedure, the optimum extracting solvent volume of 10µL was injected into the samples. The extraction efficiency was obtained using the equation:

Extraction Efficiency (EE)= (Norg/N aq)/100

= [(C org/V org)/ (Caq/Vaq)]/100

= PF x (Vorg/Vaq)/100

Preconcentration factor (PF) is the analyte concentration ratio in the extract rich with diluted surfactant which is ready for determination. Enrichment factor (EF) is the ratio of the final concentration of analyte in organic phase/ initial concentration of analyte in sample solution. The DLLME procedure was validated to test its suitability in testing BTEX in water samples. For analytical result to be adequate for its intended use, it must be satisfactory and consistent such that any result based on it can be taken with confidence.

Table 4.3 presents comparison between data obtained from this study with other microextraction methods from literature. This study results in comparison with other microextraction methods displayed in Table 5.3 show that the extraction method demonstrated good precision, linearity, detection limit and enrichment factor for the extraction of the analysis.

Table 5.3: Comparison of the proposed method with literature (AlSalka Y et al,2010).

Sample preparation methodology	LLOD μg.L^{-1}	LLDR μg.L^{-1}	RSD %	Linearity range μg.L^{-1}	Ref
HS-SPME-GC-FID	0.01-0.06	0.15-6.2	5.3-8.4	0.1-100	Wang YH et al, 2008
SPME-GC-FID	0.005-0.026	0.05-0.7	0.5-11.6	0.5-50	Zhou QX et al, 2009
DLLME-GC-FID	0.004–0.09	0.008–4.5	3.0-5.2	0.07-3-5	Assadi Y et al, 2010
DLLME-GC-MS	0.1–0.2	/	/	/	Bernardo MS et al,2009
LLE-GC-FID	1.8-5.4	2.5-250	90.0–107	4.7-6.5	Farhadi K et al, 2010
DLLME-GC-FID	0.04-0.1	0.13-0.46	1.5-3.9	6.4-12.5	This work

4.1.2 EFFECT OF VOLUME OF EXTRACTING SOLVENT

The effect of extracting solvent volume was studied using the range of 10.0–30.0μL. During the study, as the volume increased the extraction concentration decreased resulting in lower detection in the chromatographic analysis. 10 μL produced the most efficient extraction compared to the higher volumes Fig 5.1. In this study it was discovered that lower volumes have a tendency of increasing the extraction efficiency and hence enable the analytes to be detected during chromatographic analysis. With increasing extracting solvent volume, the organic phase attained by centrifugation increases resulting in the decrease in the target analyte concentration in the organic phase thereby decreasing the enrichment factor (EF). According to a study conducted by (Faraji H et al; 2013), on the effect of extracting solvent, the extraction efficiencies decreased with increasing volume of the extracting solvent. In the same study, the effect of the volume of the extracting solvent on the signal of GC-FID was investigated between 10 μL to 40 μL. The results showed that the analytical signal of toluene and benzene increased between 10 μL and 20 μL. The extraction efficiency for the xylenes and ethylbenzene decreased with the increase in extracting solvent volume. 20 µL was selected as the optimal volume of the solvent. A range of BTEX sample solutions were studied using 1.0ml acetone containing a variety of different extraction solvent volumes to get a sedimented phase of 5.0 µL. 10.0 µL and 25. 0 µL of 1,1,2 trichloro-trifluoroethane and carbon disulfide (CS_2). The average EF and standard deviation for the chosen solvents were determined. A higher EF was obtained for carbon disulphide (185.1-614.6) compared to (104.4-555.7) for 1,1,2 trichloro-trifluoroethane.

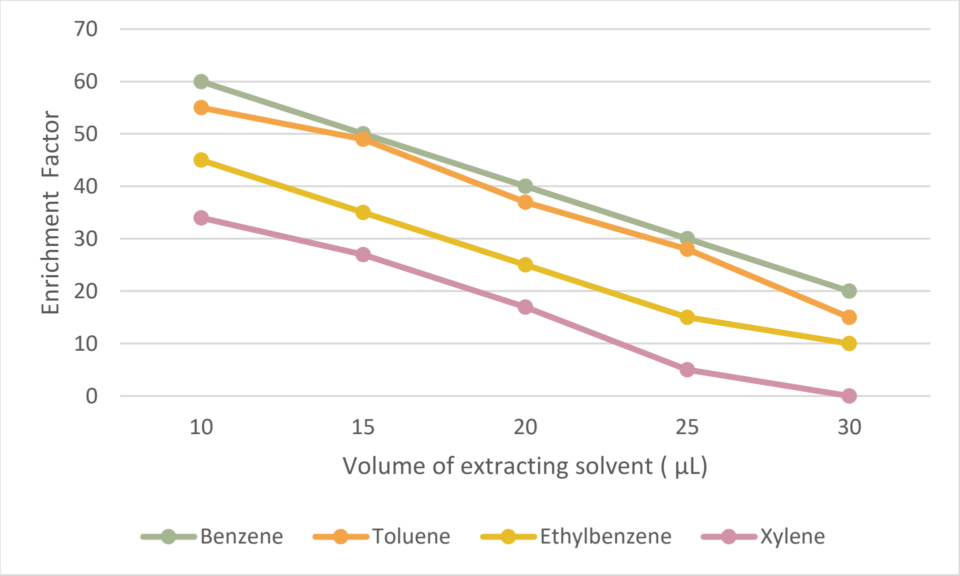


Fig 5.1: Effect of volume of extracting solvent

4.1.3 EFFECT OF EXTRACTING SOLVENT TYPE

In this study four solvents were evaluated, such as chloroform, dichloromethane, cyclohexane, and n-hexane. 10 µL of each solvent was used and injected into the sample containing BTEX. n-Hexane was found to be the best extraction solvent during the study Fig 5.2. The choice of solvent depends on the affinity for the analytes and its immiscibility in water. The solvent should have good extraction properties for the analytes, low water solubility, a higher density than water, and good chromatographic performance. In the DLLME procedure, the extraction solvent is centrifuged and hence its density should be distinct from that of water. According to literature, (Hasheminasab K.S; 2014), selection of extracting solvents considered the capability to extract, chromatographic behaviour and the formation of a cloudy state when a disperser is added.

Traditionally, heavier extractions solvents, such as chloroform and dichloromethane were used, and these were more toxic and not appropriate for analytes that are non-polar. In another study by (Sharifi V et al; 2015), solvents such as carbon tetrachloride (CCl_4), chlorobenzene, carbon disulfide and tetrachloride are used in the DLLME methodology. However, because of their toxic and hazardous nature to researchers and the environment, they are no longer considered.

Currently, less toxic extraction solvents such as n-heptane, cyclohexane and n-hexane are being used. n-Hexane has proved to have the highest extraction efficiency than n-heptane.

The study conducted by (Faraji H, et al;2013) suggests that solvents chosen must have low solubility, low volatility in water and analytes must essentially be soluble in it. n- hexadecane 1 undecanol, 2 -dodecanol, 1 dodecanol, n-hexadecane and 1- bromohexadecane were evaluated during the study. According to the study, 2-dodecanol and 1- dodecanol provided a higher extraction efficiency for the greatest number of analytes attributed to their better chromatographic behaviour, low vapour pressure and low melting point. These factors are also critical in choosing the best and most efficient extracting solvent. In another study, (Rezaee M et al, 2006), studied a range of sample solutions using 1.0 ml acetone containing a variety of different extraction solvent volumes to obtain a sedimented phase volume of 25.0 μL. 13.0 μL and 8.0 μL of CS_2, CCl_4 and C_2Cl_4 respectively. The results showed that C_2Cl_4 had the greatest extraction efficiency of (60.3-111.3%) compared to CCl_4 (24.6-74.8%) and CS_2 (16.7-48.5%). As a result, CCl_4 was chosen as the extraction solvent. The average EF and standard deviation for the chosen solvents were determined. A higher EF was obtained for carbon disulfide (185.1-614.6) compared to (104.4-555.7) for 1,1,2 trichloro-trifluoroethane. Thus, extraction solvent volume used can influence the organic phase collected above the aqueous phase after extraction, extraction efficiency and repeatability of results. When the extraction solvent was increased, the analytical signal of volume from 30 to 40 μL increased and then decreased.

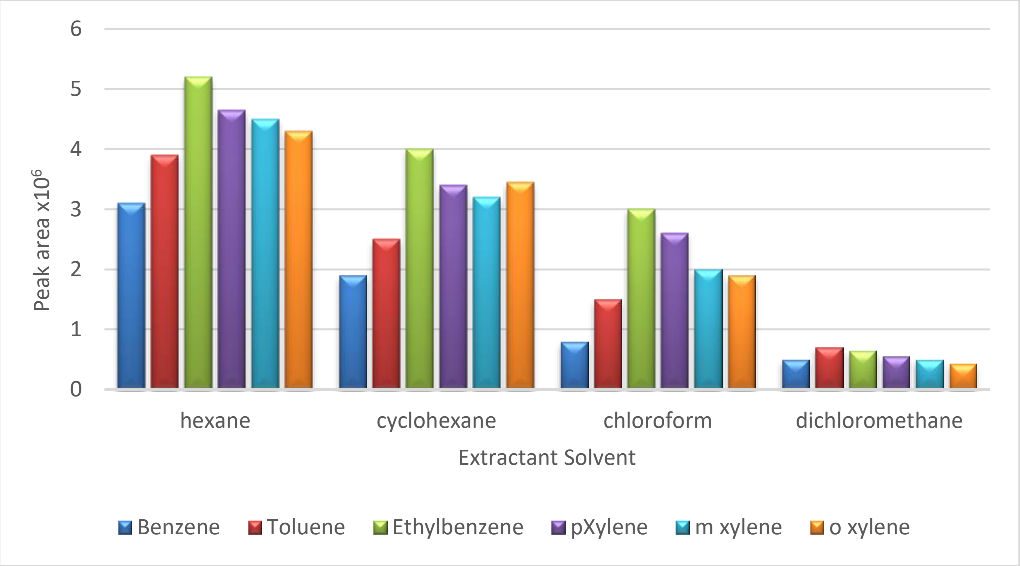


Fig 5.2: Effect of extracting solvent type

4.1.4 EFFECT OF DISPERSION SOLVENT

The following disperser were used acetone, acetonitrile, ethanol, and methanol. The type of disperser chosen is based on miscibility between the extractor and the aqueous phase. (Hasheminasab K.S et al; 2014). In a study conducted by (Rezaee M et al, 2006), a range of sample solutions were investigated using 1.00 ml of disperser solvent containing 8.0 µL of C_2Cl_4 extraction solvent. The recoveries of BTEX obtained by using acetonitrile, methanol and acetone as disperser solvents ranged from 58.5-102,6%, 66.3-105.3% and 60.3-111.3% respectively. These results illustrate that the range of recoveries using diverse disperser solvents are not different, hence acetone was chosen, because of its low cost and toxicity. In a study by (Assadi Y et al, 2010), a range of sample solutions were analysed, using 1.00 ml of a disperser phase containing 25.0 µL of carbon disulfide as an extraction solvent. The results obtained from the literature study showed that, methanol contained an impurity of interest and acetone baseline was overcrowded hence acetonitrile was selected as the disperser solvent.

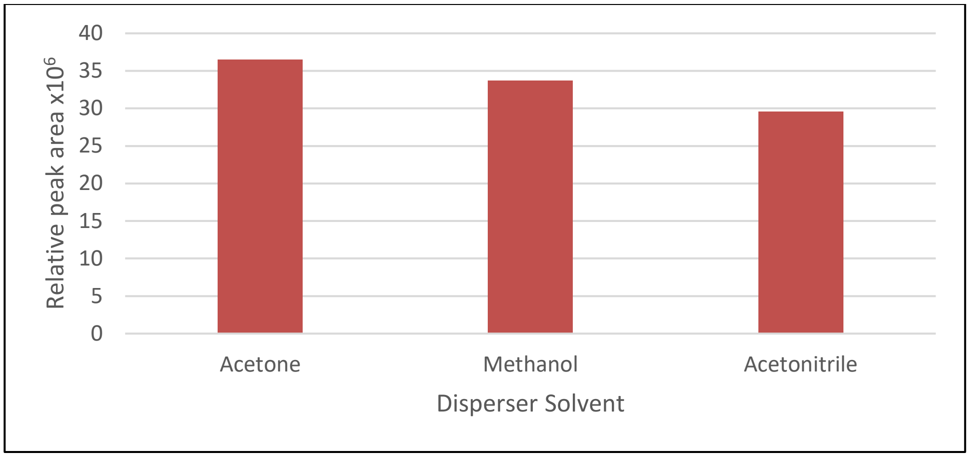


Fig 5.3: Effect of dispersion solvent

4.1.5 EFFECT OF VOLUME OF THE DISPERSER SOLVENT

Volume of acetone the disperser solvent was varied between 0.5 ml -2.00ml. The study proved that 1.00ml to be more efficient in dispersing the analytes.

Disperser solvent is also an important parameter which affects extraction efficiency. Ideal volume results in the formation of a cloudy solution depending on the volume of the extraction solvent. 0.5 μL volume of the disperser solvent failed to cause the formation of cloudy solutions because of the inability to disperse the extraction solvent properly. (Rezaee M et al, 2006) studied the effect of disperser volume where the experimental conditions were static. However, volumes of disperser solvents i.e., acetone ranged between 0.50-2.00 ml containing the volume of C_2Cl_4 7.3,8.0,10.3 and 12.4 μL respectively. Under these experimental settings the sedimented phase volume was fixed at 5.0 ± 0.2 μL. The results illustrate that the extraction efficiency increased and then decreased as the volume of acetone increased. From the current study, it seems that the low volume of disperser solvent i.e., acetone results in a cloudy state not being formed well. This decreases the extraction recovery. High volumes of acetone increase the solubility of poly aromatic hydrocarbons in water. The volume of acetone selected as the optimal volume is 1.00 ml. In a study conducted by (Assadi Y et al, 2010), the effect of acetonitrile volume was investigated over a series of volumes between 0.50-2.00ml, it was noted that the range of volumes led to differences in the volume of the sedimented phase. Therefore, it is difficult to consider the influence of acetonitrile volume on extraction efficiency using DLLME method independently. Varying volume of the disperser solvent which led to the following variation: size of droplets, volume of collected organic phase and microextraction ranged between 31-173 for all BTEX compounds. These factors are effective on extraction efficiency. It is, therefore, necessary to evaluate the volume effect of volume of solvent of disperser.

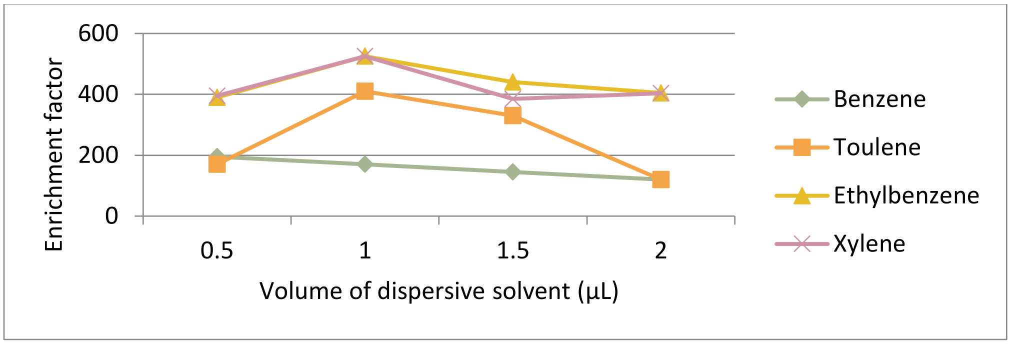


Fig 5.4: Effect of volume of the disperser solvent

4.6 APPLICATION TO REAL SAMPLES

To assess the applicability of the proposed method it was applied to real water samples. The results demonstrated that the environmental water samples contained trace quantities of BTEX compounds. Benzene, toluene, xylene, and ethylbenzene at levels of 2.67, 1.92, 0.26 and 0.41 μg. L−1, respectively as illustrated in Table 5.1 below. The results also demonstrate that effluent matrix has little effect on DLLME GC-FID method. DLLME can also be combined with MS, ICP-OES and other instruments although the method of interest was used with GC-FID. Chromatograms obtained from GC-FID for real water samples contaminated with BTEX i.e., sample 1 to 4 i.e., Fig 5.5 to Fig 5.8 are shown below. The BTEX symbol on the chromatograms represents the analytes of interest benzene, toluene, ethylbenzene, and xylene. The liner range was satisfactory and the r2 values were greater than 0.99 for all BTEX compounds. The values, show that the partition process is reproducible using these conditions, hence indicating that the method quantitative for these compounds.” The values are below 4.10% hence precision of the method is acceptable.”

Table 5.1 Results obtained from the sample

CONCENTRATION µg. L^{-1}						
Analyte	Sample1 µg. L^{-1}	Sample 2 µg. L^{-1}	Sample 3 µg. L^{-1}	Sample 4 µg. L^{-1}	Mean µg. L^{-1}	Literature
Benzene	2.7	1.98	3.5	2.5	2.67	0.1-1.8
Toluene	1.9	1.66	2.8	1.32	1.92	1-100
Ethylbenzene	0.8	0.2	1.24	0.63	0.41	0.1-1.1
Xylene	0.4	0.09	0.96	0.4	0.26	0.1-0.5

Literature ref:(Rahmawati S, 2018)

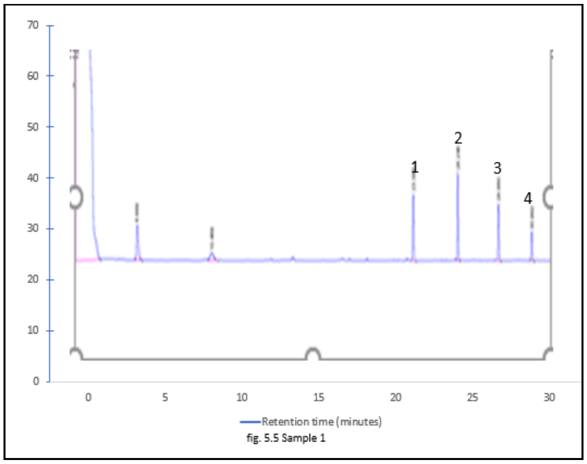
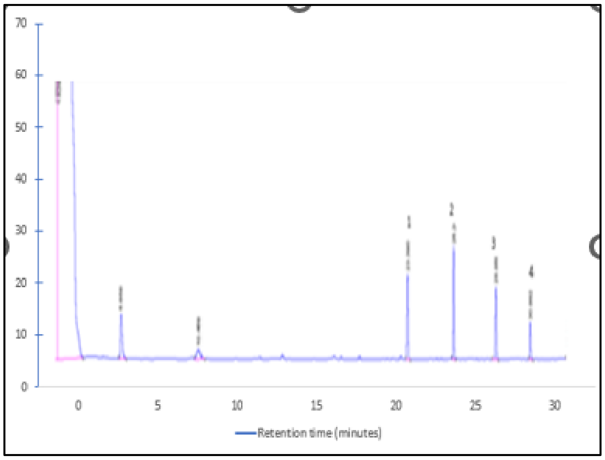


fig. 5.5 Sample 1



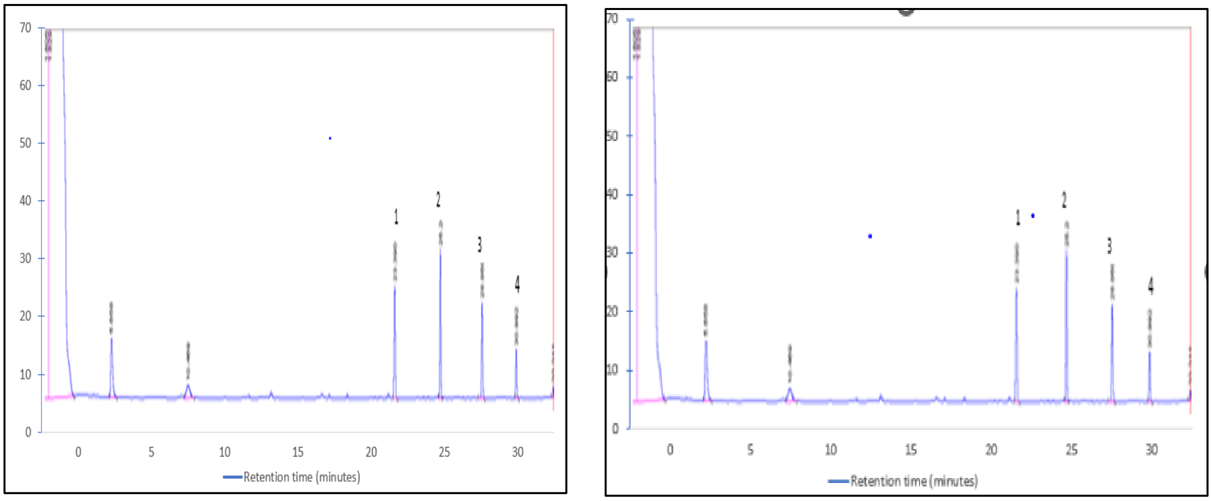


Fig 5.5-5.8: Chromatogram obtained after extraction of BTEX from environmental samples. The analytes were obtained as follows: - 1. benzene, 2. toluene, 3. ethylbenzene, 4. Xylene

4.7 CONCLUSION

This study was essential for the validation of the analytical method for determining BTEX in water samples. It was found that this is a specific, sensitive, accurate, linear, and exact method with recovery proportions ranging between 96% to 98% for the analytes. Hence, the analytical method conforms to the acceptance criteria and the outcomes obtained with the application of this method will be very reliable. It was possible to demonstrate that the method is a robust method for BTEX determination. This research allows determining in an easy and precise way a high reliability level, the concentration of volatile organic compounds (BTEX type) in samples taken in the environmental. Sample preparation time was minimized without affecting the sensitivity of the methods. Good linearity and low detection limit were obtained easily through the method.

It is evident that DLLME can be used as a preconcentration as well as extraction method for the analysis of BTEX in water samples.

In this study, GC-FID was used combined with DLLME for the determination of BTEX in water samples. The performance of this method in the extraction of BTEX from water was tested and was satisfactory. This is because DLLME results showed good repeatability, high enrichment, and high recovery factors in a short time. There are many factors that affect the extraction process which include the selection of an appropriate extraction solvent, time of extraction, an effective disperser solvent, extraction solvent volume and the disperser solvent volume. It is vital to optimize these parameters to obtain good recovery strategies. The comparison of this new method with other traditional methods such as LPME and SPME demonstrated that DLLME is efficient, inexpensive, simple, and fast.

The results from the developed method can be applied routinely and easily for screening BTEX compounds at trace concentrations in different water samples.

4.8 RECOMMENDATIONS

The following are recommendations for future studies in hydrocarbons contaminants.

- It would be beneficial to conduct a study to determine the effect of heavy metals on BTEX contaminated land.
- To determine soil type and conditions that affect the spread of the contamination in the area study
- To determine the transportation of petroleum pollutants in different seasons.
- The effect of acid mine drainage on petroleum contaminated land in Johannesburg South Africa.

4.9 REFERENCES

1. Alexander M. (1995). How toxic are toxic chemicals in soil? Environmental Science and Technology, Vol. 29, No. 11, pp. 2713–2717

2. Alexander M. (2000). Aging, bioavailability, and overestimation of risk from environmental pollutants. Environmental Science and Technology, Vol. 34, No. 20, pp. 4259–4265,

3. AlSalka Y, Karabet F, Hashem S (2010), Development and optimisation of quantitative analytical method to determine BTEX in environmental water samples using HPLC-DAD, Article in Analytical Methods, Vol.2 pp 1026-1035. DOI: 10.1039/c0ay00285b

4. Assadi Y, Ahmadi F, Hossieni MRM (2010) Chromatographia71:1137–1141

5. Anais da Academia Brasileira de Ciências (2018) 90(1 Suppl. 1): 803-824 (Annals of the Brazilian Academy of Sciences) Printed version ISSN 0001-3765 / Online version ISSN 1678-2690 http://dx.doi.org/10.1590/0001-3765201820170513.

6. Baker, R.S. & Moore, A.T. (2000). Optimizing the effectiveness of in situ bioventing. Pollution Engineering, Vol. 32, No. 7, pp. 44–47

7. Balba M.T.; Al-Awadhi, N. & Al-Daher, R. (1998). Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. Journal of Microbiological Methods, Vol. 32, No. 2, pp. 155–164,

8. Barberes GA, Pena dos Reis R, Spigolon ALD, Fonseca PE, Bandeira de Mello C, Barata MT, (2018). Groundwater Natural Contamination by Toluene in Beja and Faro Districts, Portugal. Geosciences 8(1), **https://doi.org/10.3390/geosciences8010009**

9. Bekins, B.A., I.M. Cozzarelli, E.M. Godsy, E. Warren, H.I. Essaid, and M.E. Tuccillo. 2001. Progression of natural attenuation processes at a crude oil spill site: II. Controls on spatial distribution of microbial populations. Journal of Contaminant Hydrology 53, no. 3–4: 387–406.

10. Bekins B.A., I.M. Cozzarelli, and G.P. Curtis. 2005. A simple method for calculating growth rates of petroleum hydrocarbon plumes. Groundwater 43, no. 6 pp. 817–826

11. Bing chen, Wang Shaoping, Kuang Hongbo, Shao Lei, Wang Huihui, Wang Bamgbose, and Anderson, (2020). Anaerobic-petroleum degrading bacteria: Diversity and biotechnological applications for improving coastal soil, Ecotoxicology and Environmental Safety, https://doi.org/10.1016/j.ecoenv.2021.112646

12. Brassington, K.J.; Hough, R.L.; Paton, G.I.; Semple, K.T.; Risdon, G.C.; Crossley, J.; Hay, I.; Askari, K. & Pollard, S.J.T. (2007). Weathered Hydrocarbon Wastes: A Risk Management Primer. Critical Reviews in Environmental Science and Technology, Vol. 37, No. 3, pp. 199-232,

13. Bowers R.L., Smith J.W.N., 2014. Constituents of potential concern for human health risk assessment of petroleum fuel releases. Q. J. Eng. Geol. Hydrogen.Vol. 47 pp. 363-372. http://dx.doi.org/10.1144/qjegh2014-005

14. Burland S.M. & Edwards, E.A. (1999). Anaerobic benzene biodegradation linked to nitrate reduction. Applied and Environmental Microbiology, Vol. 65, No. 2, pp. 529-533.

15. Chauhan A, Goyal MK, Chauhan P (2014) GC-MS Technique and its Analytical Applications in Science and Technology. J Anal Bioanal Tech 5: 222 doi:10.4172/2155-9872.1000222

16. CL:AIRE, 2011. A framework for assessing the sustainability of soil and groundwater remediation: Annex 1 - The SuRF-UK indicator set for sustainable remediation assessment. SuRF-UK Report. CL:AIRE, London. http://www.claire.co.uk/index.php?option=com_phocadownload&view=file&id=262:initiatives&Itemid=78

17. Cozzarelli I.M, Schreiber M.E. Erickson M.L, and Ziegler B.A 2016 Arsenic Cycling in Hydrocarbon Plumes: Secondary Effects of Natural Attenuation; Vol. 54, No. 1 pp.35–45)

18. Cozzarelli I.M., B.A. Bekins, M.J. Baedecker, G.R. Aiken, R.P. Eganhouse, and M.E. Tuccillo. 2001. Progression of natural attenuation processes at a crude-oil spill site: I. Geochemical evolution of the plume. Journal of Contaminant Hydrology 53, no. 3–4: pp. 369–385.

19. Cozzarelli I.M., J.R. McKelvie, and A.L. Baehr. 2014. Volatile hydrocarbons and fuel oxygenates. In Treatise on Geochemistry, 2nd ed., ed. H.D. Holland and K.K. Turekian, pp. 439–480. Oxford, UK: Elsevier.

20. David F., Tienpont B, Sandra P, 2003, Stir-bar sorptive extraction of trace organic compounds from aqueous matrices. LCGC Europe Vol.17 No. 7 pp.2-7

21. Davis G.B., Johnston, C.D., Thierrin, J., Power, T.R. and Patterson, B.M. (1993) Characterising the distribution of dissolved and residual NAPL petroleum hydrocarbons in unconfined aquifers to effect remediation. Journal of Australian Geology and Geophysics, 14(2/3), pp.89-94.

22. Demeestere K., J. Dewulf, B. D. Witte, and H. V. Langenhove. 2007. Sample preparation for the analysis of volatile organic compounds in air and water matrices. J. Chromatogr. A Vol1153: pp.130–44.

23. Diao C.P, Wei C.H, Feng C.H (2012) Rapid determination of Benzene Derivatives in Water samples by trace volume solvent DLLME prior to GC-FID; DOI 10.1007/s10337-012-2215-7; Chromatographia (2012) Vol. 75 pp. 551–555

24. Duan X, Li Y (2017). Sources and Fates of BTEX in the General Environment and Its Distribution in Coastal Cities of China. Journal of Environmental Science and Public Health Vol 1 No. 2, doi: 10.26502/JESPH.009

25. Eom I., A. Tugulea, and J. Pawliszyn. 2008. Development and application of needle trap devices. J. Chromatogr. A Vol. 1196 pp. 3–9.

26. Fayemiwo OM , Daramola MO, Moothi K (2017). BTEX compounds in water – future trends and directions for water treatment, Vol. 43 No. 4. pp. 1816-7950

http://dx.doi.org/10.4314/wsa.v43i4.08,

26. Farhadi K, Maleki R, Tahmasebi R (2010) Anal Lett 43:349–356, P:/Santype/Journals/TandF_Production/LANL/v43n2/lanl432763/lanl432763.3d; Microextraction of BTEX compounds from water samples using Olive oil droplets. Anal Vol.43 pp. 349–356,

27. Faraji H, Feizbakhsh A, Helalizadeh M (2013), Modified dispersive liquid-liquid microextraction for pre-concentration of benzene, toluene, ethylbenzene and xylenes prior to their determination by GC-FID. Microchim Acta Vol 180 pp. 1141–1148 DOI 10.1007/s00604-013-1037-0

28. Farah Wahidah Mohd Hassan,Muggundha Raoov,Sazlinda Kamaruzaman,Mohd Marsin Sanagi,Nao Yoshida,Yuichiro Hirota,Norikazu Nishiyama,Noorfatimah Yahaya (2018). Separation Science, Vol. 14. https://doi.org/10.1002/jssc.201800326

29. Ferenc A.Z, and Biziuk M (2006), Solid phase extraction technique-trends, opportunities and applications. Polish J. of Environ. Stud. Vol. 15, No. 5 pp. 677-690

30. Feris K., Hritova, K., Cebreyesus, B., Mzckay, D., and Shallow S.K.A; BTEX and MTBE Contaminated Aquifer Supports Microbial Community. Kluwer Academic Publishers, Netherlands. 2004.

31. Fisher A., Oehm, C., Selle, M., and Werner, P. Biotic and Abiotic Transformations of methyl tertiarybutyl ether. SpringerLink. 2005.

32. Gogoi B.K., Dutta, N.N., Goswami, P., and Krishna Mohan, T.R., 2003, A case study of bioremediation of petroleum-hydrocarbon contaminated soil at a crude oil spill site: Advances in Environmental Research, Vol. 7, no. 4, pp. 767-782.

33. Godsy E.M., Warren, Ean, Cozzarelli, I.M., Bekins, B.A., and Eganhouse, R.P., 1999, Determining BTEX biodegradation rates using in-situ microcosms at the Bemidji site, Minn., in Morganwalp, D.W., and Buxton, H.T., eds., U.S. Geological Survey Toxic Substances Hydrology Program Technical Meeting, Charleston, S.C., 1999, Proceedings: U.S. Geological Survey Water-Resources Investigations Report 99-4018C, pp. 211-222.

34. Gonzalo A. L., J. E. Sanchez-Uria, E. S. Garica, and A. S. Medel. 2008. Critical comparison of automated purge and trap and solid-phase microextraction for routine determination of volatile organic compounds in drinking waters by GC-MS. Vol. 74 pp.1455–62.

35. Hardisty P.E., Wheater, H.S., Johnston, P.M., Bracken, R.A., 1998. Behaviour of light immiscible liquid contaminants in fractured aquifers. Geotechnique, Vol. 48 pp.747-760. http://dx.doi.org/10.1680/geot.1998.48.6.747

36. Hasheminasab K.S, Fakhari A.R, Baghdad M, 2013. Trace Determination of Petroleum Pollutants in Water Samples by Dispersive Liquid–Liquid Microextraction Method, Clean – Soil, Air, Water 2014, Vol. 42 No. 8, pp. 1106–1114, DOI: 10.1002/clen.201300044

37. Headley J.V., Gong Y, Barbour S.L, Thring R. 2000. 'An Evaluation of the Ideality of Benzene, Toluene, Ethylbenzene, and Xylene on Activity Coefficients in Gas Condensate and the Implications for Dissolution in Groundwater." Canadian Water Resources Journal, Vol.25 No. pp. 67-79

38. Hsia KF, Chen CC, Ou JH, Lo KH, Sheu YT, Kao CM (2021). Treatment of petroleum hydrocarbon-polluted groundwater with innovative in situ sulfate-releasing biobarrier. Journal of Cleaner Production. https://doi.org/10.1016/j.jclepro.2021.126424

39. In- Yong Eom, 2011 Estimation of Partition Coefficients of Benzene, Toluene, Ethylbenzene and p-Xylene by Consecutive Extraction with Solid Phase Microextraction. Korean Chem. Soc. 2011, Vol. 32, No. 5 pp. 1463

40. ITRC (Interstate Technology & Regulatory Council), 2009a. Evaluating LNAPL remedial technologies for achieving project goals. Interstate Technology and Regulatory Council, LNAPLs Team. http://www.itrcweb.org/guidance/getdocument?documentid=48

41. Ivančev-Tumbas, I.; Tričković, J.; Karlović, E.; Tamaš, Z.; Rončević, S.; Dalmacija, B.; Petrović, O. & Klašnja, M. (2004). GC/MS-SCAN to follow the fate of crude oil components in bioreactors set to remediate contaminated soil. International Biodeterioration and Biodegradation, Vol. 54, No. 4, pp. 311-318,

42. Ji, J., C. Deng, W. Shem, and X. Zhang. 2006. Field analysis of benzene, toluene, ethylbenzene, and xylene in water by portable gas chromatography–microflame ionization detector 225 combined with headspace solid-phase microextraction. Vol. 69: pp.894–99.

43. Johnson, P.C., Lundegard, P., Liu. Z., 2006. Source zone natural attenuation at petroleum hydrocarbon spill sites: I. Site-Specific Assessment Approach. Groundwater Monit. R., Vol 26, No. 4 pp. 82-92. http://dx.doi.org/10.1111/j.1745-6592.2006.00114.x

44. Johnston C.D., 2010. Selecting and assessing strategies for remediating LNAPL in soils and aquifers. CRC for contamination assessment and remediation of the environment, CRC Care Technical Report No.18. http://www.crccare.com/publications/technical-reports/technical-reports.

45. Kamal M.A, Klein P, 2010, Estimation of BTEX in groundwater by using gas chromatography–mass spectrometry, Saudi Journal of Biological Sciences.

46. Kennedy, Lonnie, Everett, Jess, and Gonzales, James, 2000, Aqueous and mineral intrinsic bioremediation assessment (AMIBA) protocol: Brooks Air Force Base, Tex., Air Force Center for Environmental Excellence, Technology Transfer Division, pp. 286

47. Konečný F, Boháček Z, Müller P, Kovářová M, Sedláčková I, 2003; Contamination of soils and groundwater by petroleum hydrocarbons and volatile organic compounds – Case study: Elslav Brno; Bulletin of Geosciences, Vol. 78, No. 3, pp. 225–239,

48. Lee M. R., C. M. Chang, and J. Dou. 2007. Determination of benzene, toluene, ethylbenzene, 235 xylenes in water at sub-ngl(1) levels by solid-phase microextraction coupled to cryo-trap gas chromatography-mass spectrometry. Chemosphere Vol. 69 pp.1381–87

49. Loeher R.C.; Mc Millen, S.J. & Webster, M.T. (2001). Predictions of biotreatability and actual results: soils with petroleum hydrocarbons. Practice periodical of hazardous, toxic, and radioactive waste management, Vol. 5, No. 2, pp. 78–87

50. Maletić S, Dalmacija B and Rončević S; 2013 Petroleum Hydrocarbon Biodegradability in Soil – Implications for Bioremediation

51. Mahgoub H. A. (2016). Extraction techniques for determination of polycyclic aromatic hydrocarbons in water samples. International Journal of Science and Research, Vol. 5, pp.268-272. doi:10.21275/v5i1.nov152648

52. Mercer J.W., Cohen, R.M., 1990. A review of immiscible fluids in the subsurface: properties, models, characterization and remediation. J. Contam. Hydrol., Vol. 6 No.2 pp.107-163. http://dx.doi.org/10.1016/0169-7722(90)90043-G

53. McMichael T. 2003. Environmental Impact Assessment of Petrol Usage. Aarhus University, Denmark.

54. Molins S., Mayer, K.U., Amos, R.T., Bekins, B.A., 2010. Vadose zone attenuation of organic compounds at a crude oil spill site – interactions between biogeochemical reactions and multicomponent gas transport. J. Contam. Hydrol., Vol. 112, pp. 15-29. http://dx.doi.org/10.1016/j.jconhyd.2009.09.002

55. Mostafa Khajeh,Ali Akbar Moosavi-Movahedi,Mohammad Shakeri,Fatemeh Musavi Zadeh,Ahmad Khajeh,Mousa Bohlooli (2015). Dispersive solid phase extraction combined with dispersive liquid–liquid extraction for the determination of BTEX in soil samples: ant colony optimization–artificial neural network. Chemometrics, 7 https://doi.org/10.1002/cem.2706

56. National Research Council, 1993, In situ bioremediation: when does it work? Washington, D.C., National Academy Press, pp. 207

57. Newell C., Acree, S., Ross, R., and Hulling, S, 2006 Groundwater Issues. Environmental Protection Agency.

58. Nilanjana D. & Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotechnology Research International, Vol. 2011, pp. 1-13,

59. O M Fayemiwo1, MO Daramola2 and K Moothi1 2017;

*http://dx.doi.org/10.4314/wsa.v43i4.08, ISSN 1816-7950 (Online) = Water SA Vol. 43 No. 4

October 2017SSN 1816-7950 (Online) = Water SA Vol. 43 No. 4

60. Oostrom M., Hofstee, C., Wietsma, T.W., 2006. Behavior of a viscous LNAPL under variable water table conditions. Soil Sediment Contam. Vol. 15 No. 6 pp. 543-564. http://doi:10.1080/15320380600958976

61. Patrycja Makoś,André Fernandes,Grzegorz Boczkaj (2018). Method for the simultaneous determination of monoaromatic and polycyclic aromatic hydrocarbons in industrial effluents using dispersive liquid–liquid microextraction with gas chromatography–mass spectrometry. Separation Science,28, https://doi.org/10.1002/jssc.201701464

62. Phelps C.D., Young, L., and Geng, L; 1999, Anaerobic Biodegradation of BTEX and Gasoline in Various Aquatic Sediments. Kluwer Academic Publishers, Netherlands. 1999.

63. Pollard, S.J.T.; Hough, R.L.; Kim, K.; Bellarby, J.; Paton, G.I.; Semple, K.T. & Coulon, F. (2008). Fugacity modelling to predict the distribution of organic contaminants in the soil: oil matrix of constructed biopiles. Chemosphere, Vol. 71, No. 8, pp. 1432–1439,

64. Potter T.L., and Simmons, K.E., 1998, Composition of petroleum mixtures: Amherst, Mass., Amherst Scientific Publishers, Total petroleum hydrocarbon criteria working group series, Vol. 2, pp. 102

65. Praseeja A.V and Sajikumar N (2020). LNAPL Migration in Vadoze Zone and Its Prevention Using Natural Fiber. JOURNAL OF NATURAL FIBERS https://doi.org/10.1080/15440478.2020.1848701

66. Quingley A, Cummins W, Connolly D, 2016, Dispersive Liquid-Liquid Microextraction in the Analysis of Milk and Dairy Products: A Review, Journal of Chemistry Volume 2016, Article ID 4040165, 12 pages http://dx.doi.org/10.1155/2016/4040165

67. Rahmani M., Kaykhaii, M., Ghasemi, E., & Tahernejad, M. (2015). Application of In-Syringe Dispersive Liquid–Liquid Microextraction and Narrow-Bore Tube Dispersive Liquid–Liquid Microextraction for the Determination of Trace Amounts of BTEX in Water Samples. Journal of chromatographic science, Vol. 53 pp.1210.

68. Ramphisa P. and Meeuwis, J The use of petroleum products in gold mining—environmental impacts and cost implications. World Gold Conference 2009, The Southern African Institute of Mining and Metallurgy, 2009

69. Rasmussen K.E, Pedersen-Bjergaard S, Gjelstad A, 2012, Hollow Fiber Liquid-Phase Microextraction, DOI:10.1016/B978-0-12-381373-2.00054-5

70. Suphia Rahmawati; Any Juliani; Wahyuningtyas Perwita Sari; Azkiyatul Bariroh (2018). Investigation of Groundwater Pollution by Petroleum Hydrocarbon from Gas Station in Yogyakarta, Indonesia. urnal Sains dan Teknologi Lingkungan, Volume 10, No. 1, pp. 67-76

71. Rivett M.O., Wealthall, G.P., Dearden, R.A., McAlary, T.A., 2011. Review of unsaturated-zone transport and attenuation of volatile organic compound (VOC) plumes leached from shallow source zones. J. Contam. Hydrol., Vol. 123 pp.130-156.http://dx.doi.org/10.1016/j.jconhyd.2010.12.013

78. Sarafraz-Yazdi, A., A. H. Amiri, and Z. Es'haghi. 2008. BTEX determination in water matrices using HF-LPME with gas chromatography-flame ionization detector. Chemosphere Vol. 71 pp. 671–76.

79. Sarafraz-Yazdi A, Piri moghadam H, Es'haghi Z, Sepehr S (2010) Comparative study of the three sol–gel based solid phase micro extraction fibers in extraction of BTEX from water samples using gas chromatography-flame ionization detection. Anal Methods Vol 2 pp. 746–752

80. Salminen J.M.; Tuomi, P.M.; Suortti, A. & Jørgensen, K.S. (2004). Potential for Aerobic and Anaerobic Biodegradation of Petroleum Hydrocarbons in Boreal Subsurface. Biodegradation, Vol. 15, No. 1, pp. 29-39,

81. Semple K.T, Reid R.J, Fermor T.R, 2001; Impact of Completing Strategies on the treatment of soils contaminated with organic pollutants. Environmental Pollution.

82. Serrano A., and M. J. Gallego. 2004. Direct screening and confirmation of benzene, toluene, ethylbenzene, and xylenes in water. J. Chromatogr. A Vol. 1045 pp. 181–89.

83. Sieg, K., E. Fries, and W. Puttmann. 2008. Analysis of benzene, toluene, ethylbenzene, xylenes 250 and n-aldehydes in melted snow water via solid-phase dynamic extraction combined with gas chromatography=mass spectrometry. J. Chromatogr. A Vol. 1178 pp. 178–86

84. Semih O, and Canan K. 2015; Solid-phase extraction (SPE): Principles and applications in food samples; Acta Sci. Pol. Technol. Aliment. Vol. 15, No 1 pp. 5–15; DOI: 10.17306/J.AFS.2016.1.1

85. Spence M.J., Bottrell, S.H., Thornton, S.F., Richnow, H., Spence, K.H., 2005. Hydrochemical and isotopic effects associated with fuel biodegradation pathways in a chalk aquifer. J. Contam. Hydrol., Vol. 79 pp.67-88. http://dx.doi.org/10.1016/j.jconhyd.2005.06.003 TCEQ (Texas Commission on Environmental Quality), 2008. Risk-based NA

86. Stroud J.L, Paton G.I, Semple K.T, 2007; Microbe- aliphatic hydrocarbon interactions in soil; implications for biodegradation & remediation Journal of Applied Microbiology.

87. Stroud J.L, Paton G.I, Semple K.T, 2009; Predicting the biodegradation of target hydrocarbons in the presence of mixed contaminants in soil. Chemosphere.

88. Sarem Targuma 1 , Patrick B. Njobeh 2 and Patrick G. Ndungu (2021). Current Applications of Magnetic Nanomaterials for Extraction of Mycotoxins, Pesticides, and Pharmaceuticals in Food Commodities. Molecules Vol. 26 No. 4. https://doi.org/10.3390/ molecules26144284.

89. Thompson, K. and Nathanail, K, 2003 Chemical Analysis of Contaminated Land. Blackwell Publishing. Oxford

90. Tomlinson D.W, Thornton S.F, Thomas A.O, Leharne S.A, 2014, An Illustrated Handbook of LNAPL Transport and Fate in the Subsurface.

91. USEPA, 2013. Evaluation of empirical data to support soil vapor intrusion screening criteria for petroleum hydrocarbon compounds, EPA-510-R-13-001. http://www.epa.gov/oust/cat/pvi/PVI_Database_Report.pdf

92. U.S. Environmental Protection Agency, 1999, Monitored natural attenuation of petroleum hydrocarbons: Washington, D.C., U.S. Environmental Protection Agency, Office of Research and Development, Fact Sheet, EPA/600/F-98/021, 3 .

93. USEPA, 2009. Underground storage tank program – 25 years of protecting our land and water. EPA510-B-09-001. http://www.epa.gov/swerust1/pubs/25annrpt.htm

94. USEPA (2011). Analytical method approved for drinking water compliance monitoring of organic contaminants. U.S. Environmental Protection Agency, Washington, DC. Available at:

http://water.epa.gov/scitech/drinkingwater/labcert/upload/methods_organic.pdf

95. Usher B., Pretorius, J., Dennis, I., Javanovic, N. et al. Identification and Prioritisation of Groundwater Contaminants and Sources in South Africa's Urban Catchments. Water Research Commission, Pretoria. 2004

96. Users' Guide to Guidelines for Assessing and Managing Petroleum Hydrocarbon Contaminated Sites In New Zealand; 1999 New Zealand

97. Wiedemeier T.H., Wilson, J.T., Kampbell, D.H., Miller, R.N., and Hansen, J.E., 1995, 98. Technical protocol for implementing intrinsic remediation with long-term monitoring for natural attenuation of fuel contamination dissolved in groundwater, v. 1: San Antonio, Tex., Air Force Center for Environmental Excellence, Technology Transfer Division, Brooks Air Force Base, pp. 295

98. Williams S.D, Ladd D.E, and. Farmer J.J, 2005; Fate and Transport of Petroleum Hydrocarbons in Soil and Ground Water at Big South Fork National River and Recreation Area Tennessee and Kentucky 2002-2003

99. Xiaodong Zhang1,2and Guo H. Huang (2011). Assessment of BTEX-induced health risk under multipleuncertainties at a petroleum-contaminated site : An integratedfuzzy stochastic approach. Water Resources Research, Vol 47, W12533, http://dx.doi.org/10.1029/2011WR010682.

100. Yaghoub Assadi, Fardin Ahmadi and Mohammad R.M.H, 2010: Determination of BTEX Compounds by Dispersive Liquid–Liquid Microextraction with GC–FID; DOI: 10.1365/s10337-010-1616-8 0009-5893/10/06, Chromatographia No.11/12

101. Yanguo Teng (2021). Analysis of the LNAPL Migration Process in the Vadose Zone under Two Different Media Conditions. International journal of Environmental Research and Public Health.

102. Zuo Rui, Xiao Zhao, Jie Yang, Minghao Pan, Zhenkun Xue, Xiang Gao, Jinsheng Wang, and Yanguo Teng (2021). Analysis of the LNAPL Migration Process in the Vadose Zone under Two Different Media Conditions. International journal of Environmental Research and Public Health