DEVELOPMENT OF METHODS FOR THE ANALYSIS OF PETROLEUM CONTAMINATED SOILS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AHs	Aromatic Hydrocarbons
ASE	Accelerated Solvent Extraction
ASTM	American Society for Testing Materials
BTEX	Benzene, Toluene, Ethylbenzene and Xylenes
CL	Confidence Level
DRO	Diesel Range Organics
EPH	Extractable Petroleum Hydrocarbons
FID	Flame Ionization Detector
GC	Gas Chromatography
GRO	Gasoline Range Organics
HCs	Hydrocarbons
HS	Headspace
ICP	Inductively Coupled Plasma
IS	Internal Standard
L	Litres
LLE	Liquid- liquid Extraction
MAE	Microwave assisted extraction
MeOH	Methanol
MS	Mass Spectrometry
MTBE	Methyl Tertiary Butyl Ether
MTBE	Methyl Tertiary Butyl Ether
NMVOCs	Non-Methane Volatile Organic Compounds
PA	Peak Area
PAH	Polycyclic aromatic hydrocarbon
PCA	Principal Component Analysis
PCR	Principal Component Regression
Pht	Peak Height
PID	Photo Ionisation Detector.
PLOT	Porous Layer Open-Tubular
PLS	Partial Least Square
PPM	Part Per Million

QA	Quality Assurance
QC	Quality Control
ROGs	Reactive Organic Gases
RP	Reference point (sampling point from well head)
RSD	Relative Standard Deviation
RTW	Retention Time Window
SCOT	Support Coated Open-Tubular
SEA	Soxhlet Extraction Apparatus
SFE	Supercritical Fluid Extraction
SPME	Liquid Phase Microextraction
TCD	Thermal Conductivity Detector
TPH	Total Petroleum Hydrocarbon
TSP	Trisodium phosphate dodecahydrate
USEPA	United States Environmental Protection Agency
VOC(s)	Volatile Organic Compound(s)
WCOT	Wall Coated-Open Tubular
WH	Well Head
WOO	Waste oil Organics

ABSTRACT

Soil contamination from petroleum spills is a frequent environmental problem in the world. It is obvious that petroleum exploration has contributed immensely to the economic growth of Nigeria, but over the last few decades, the Niger Delta of Nigeria has suffered grave human health risk and ecosystem degradation resulting from oil spillages, petroleum products leakages and other involuntary effluent discharges from oil exploration activities.

This research seeks to develop and optimize GC-FID methods for the analysis of Petroleum hydrocarbons. Crude oil spillage contamination of soil from the Niger Delta was investigated 3 months after a crude oil-pipeline spillage. 47 Soil samples (300-500g) were collected at several points in the South-South Niger Delta. Control samples were taken from four unaffected sites within the vicinity of spillage with similar soil characteristics. Samples were collected at depths of 0-15 cm, 15-30 cm and 30-60 cm. The soil samples were prepared for analysis using solvent extraction methods, passed through column of sodium sulphate and Florisil® to aid in column performance, remove moisture and gross impurities. Samples were analysed using gas chromatography with a flame ionisation detector.

Penetration and migration of C10-C26 and C26-C34 hydrocarbons through the soil layers were assessed by cluster analysis to determine the spatial distribution, penetration and chemical similarity of these compounds over the contaminated area. This information is a useful guide for bioremediation purpose. It was found that total petroleum hydrocarbon concentrations varied from 9-289 $mgkg^{-1}$ topsoil, 8-318 $mgkg^{-1}$ subsoil and 7-163 $mgkg^{-1}$ at the greatest depth measured.

The results show elevated levels of total hydrocarbon contents when compared with the reference sites. Drastic steps should be taken to carefully monitor and remediate the environment. Bioremediation with plants and micro-organisms is endorsed.

DECLARATION

The work presented in this thesis was carried out in the school of Chemical Engineering and Analytical Science between November 2006 and October 2009.

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification at this or any other university or other institute of learning.

COPYRIGHT STATEMENT

DEDICATION

This research work is dedicated to my dear wife Comfort and my lovely children - Mfoniso, Nsisong, Endiong and Kuseme.

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CHAPTER 1

INTRODUCTION

1.1 Petroleum Contaminants in Soils

In the past few decades there has been an increased public awareness of environmental issues particularly when contamination of soil, water and air is involved. Globally, scientists and environmentalists are faced with the challenge of overcoming the detrimental effects of the contamination of soil, air and water. Spillages of crude oil on soil, leakages from pipelines, underground and surface fuel storage tanks, indiscriminate spills and careless disposal and mismanagement of waste and other by-products of the society, constitute the major sources of petroleum contamination in the environment.

In recent years, environmental pollution due to the increasing release of hazardous and toxic substances into the soil, water, sediment and air in Niger Delta, Nigeria has been a widespread problem. In deed soil contamination by oil exploration activities has quickly become a considerable environmental issue [1, 2, and 3]. There is little doubt that the issue of petroleum contamination of soils and water has become a topic of interest and is attracting increasing attention because of the carcinogenic, mutagenic and toxic effects. [4, 5, 6].

Future generations face the threat of the problems resulting from the effects of present irresponsible behaviour towards the environment.

Petroleum spills and leakages from source often contaminate the impacted soil and ecosystem. Petroleum (crude oil) is used to make petroleum products and they possess the potential to contaminate the environment. In fact, there are so many different chemicals in crude oil and in other petroleum products, so that it becomes practically difficult to measure each one separately. However, it

is useful to measure the Total Petroleum Hydrocarbon (TPH) amount at the chosen site.

The analytical goal for each petroleum spill site is to access the level of contamination and to efficiently and safely remove the spilled petroleum products from the soil with the aim of returning the soil back to a useable form. Attempts for complete removal may not be practically attainable either due to cost or source, then the objective is to remediate the soils to the concentration levels that will be harmless to plants, fauna, human health and the entire ecosystem [7, 8, 9, 10]. The way to handle, dispose or reuse non-hazardous petroleum contaminated soils has received attention.

1.2 Research Background

Environmental contamination by petroleum spills and petroleum products is of significant concern in the world and particularly in Niger Delta of Nigeria.

The economy of Nigeria, a most populous, black African country is largely dependent on crude oil tapped from the Niger Delta region. It is estimated that 2.4 million barrels of crude oil per day come from this region [11]. Niger Delta has a population of about 30 million people from nine of the 36 States of Nigeria [12, 13]. This region (refer to figure 1.1) is not only rich in oil but solid minerals, fish from the rivers and ocean, huge plants and variety of animals from the swampy forest vegetation. The protracted activities of oil drilling occasionally resulting in frequent spillages and gas flares has rendered the once-fertile -farm lands barren, with disappearance of vegetation, animals and fish as a result of environmental contaminants from petroleum. This situation led to poverty arising from joblessness, emergence of irate militants, and the jeopardy of human and ecological lives.

Most human, aquatic and terrestrial lives of the Niger delta are endangered which often aroused conflicts and threats of abduction of oil workers. Poor supply of electricity, water, road network, and poor health care system plagued the oil region leaving them to drink from wells, streams, rivers, lakes and most often from stagnant ponds that are extremely contaminated.

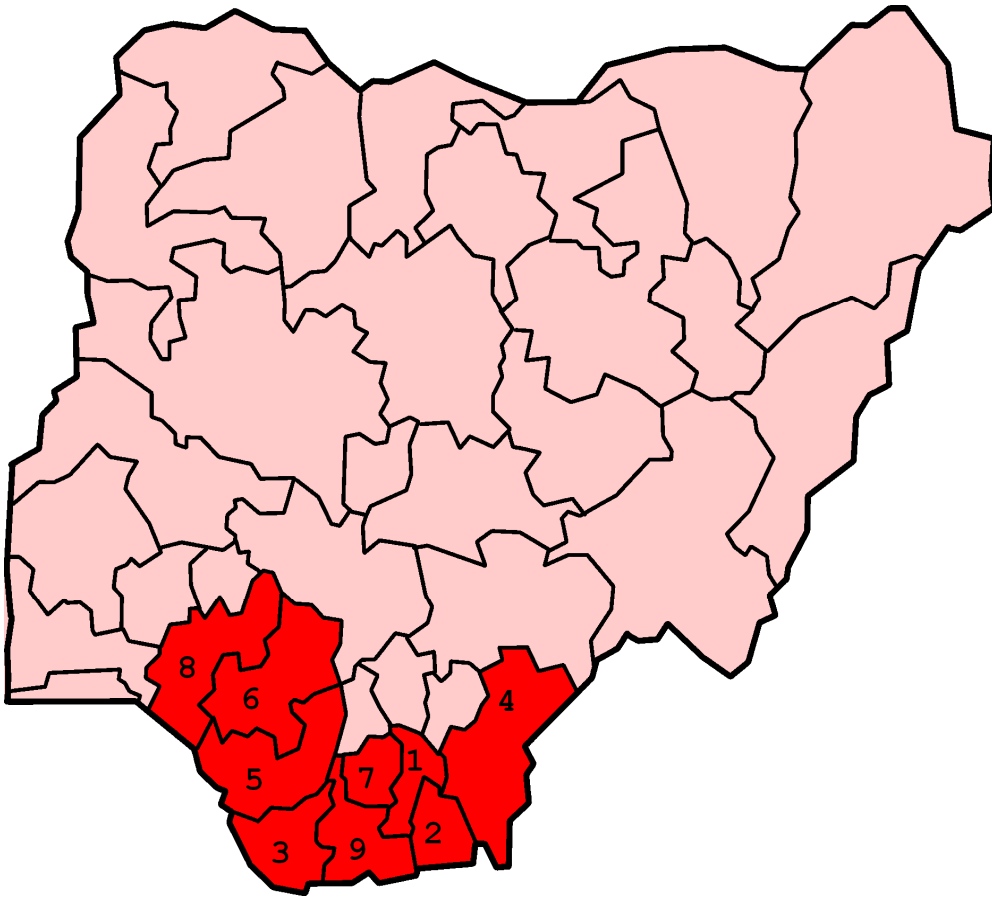

Figure 1.1 Map of Nigeria with 36 states and Niger Delta area inscribed in red [14]

KEY

Nine (9) Niger Delta States of the 36 states of Nigeria.

(I) Abia (2) Akwa Ibom (3) Bayelsa (4) Cross River

(5) Delta (6) Edo (7) Imo (8) Ondo (9) Rivers

Incessant cases of oil spillages and gas flares have left much to be desired about the human health risk and environmental deprivation of this region as shown by the pictures in figures 1.2 and 1.3 A & B below.

Figure 1.2 Crude Petroleum spill on farm land in Ikot Ada Udo village, Akwa Ibom State, South-South Niger Delta, Nigeria. The Well head is shown after recent spillage.

Figure 1.3 (A) Oil spill and broken oil pipeline fire outbreak in Elume village in Niger Delta. The thick smoke polluted the atmosphere and completely burned off the rubber plantations.
(B) Gas flares in a village in Niger Delta.

Negligence and or operational equipment failure could cause a lot of this hazard but the issue of concern is the polluted atmosphere created for animal and plant niche of this environment such as in figures 1.4 below.

Figure 1.4 Incident of Oil pipeline explosion, in Niger Delta. The picture shows a female villager running With her nose covered to avoid inhaling much of the characteristically odorous hydrocarbon gas.

1.3 Highlights of Difficulties of the Analytical Operations in Niger Delta

There are prevalent difficult situations hampering analytical operation in this area which are not exhaustive but include the following:

- **Bad and inaccessible roads**.

  The road from the nearest city, Uyo, and Port Harcourt terminates at a point beyond the sampling arena. Ferry and boats are needed to cross

from one creek/island to the next within the same environment at an average time of 40 minutes. The sampling equipment and reagents must be carried in a box or bag across to these points as vehicles may not penetrate up those zones. It takes more than two hours to drive to the end of access road from the nearest city, a distance of more than 80km.

- **Lack of constant Electricity supply**. There is no electric power supply in the sampling area or environment. Portable generator may be used when needed and could be transported there.
- **The thick rural vegetation with human conflicts.** The vegetation is thick and massive, with track roads that cannot be driven by car. Usually, this is a volatile militant region, and the natives often confront the sampling personnel for money ransom or disturb the exercise thinking operation is politically motivated. Therefore to avert this problem of harassment and possible kidnapping, the researcher, after due consultation with the chiefs was often escorted by the village vigilante groups to the site for each sampling trip. Restriction was usually placed on the site to avoid undue encroachment.
- **Sampling complexity in marshy areas**. Difficulty of location of sampling points may occur in this loamy soil and sediment. Clearing tracks and fields for space may be the option before executing the sampling plan, and this calls for more hands and sometimes the services of the villagers were employed as a better option.
- **Weather Conditions**. The sampling location which is in South-South Niger Delta, Nigeria, has two seasons a year, namely the dry and the rainy seasons. The dry season which incidentally falls in the time of this sampling, starts from November to April and is characterized by High temperature of 40°C±2. Lack of rain for about six months leaves the soil very dry and hard to dig. The rainy season commences from April to the end of October and has an average temperature of 25°C. This period is always accompanied by heavy rainfall with loose wet soil.
- **Inaccessible and poorly equipped Laboratory.** The closest standard laboratory to this sampling area is in Port Harcourt, about 120 km and takes about 3hrs to drive on road. Two laboratories within the nearest

town from the sampling site are poorly equipped and may not be used to carry out any pilot test on the analytes.

1.4 Analytes of Interest

The analytes considered for this work consist of a group of Volatile Organic Compounds (VOCs) found in Petroleum – BTEX, (C5–C9) and Extractable Petroleum Hydrocarbons (EPHs) in the Diesel Range Organics (DRO) or Fuel/Waste Oil (C10 - C40). The method defines DRO as containing carbon number C10-C28 with boiling point range between 170°C- 430°C. Gasoline and other liquid hydrocarbons cover C6-C26 with boiling points of 70°C-400°C.
These groups of VOCs/liquid hydrocarbons are notorious in soil and water contamination, hence pose serious health and ecological hazards to the native environments as described below under pollution and human impact in section 1.5.

1.5 Pollution and Human Impact

Pollution has inevitably become undesirable phenomenon of global concern. It is a known fact that industrial revolution gave birth to environmental pollution as it is today. The continuous degradation of and impending damage to our valued environment by pollution is a thing of concern and should not be overlooked. Environmental Pollution affects soil, water and air, presenting health danger to humans, aquatic life, ecosystem and harmful threat to the natural environment [15, 16]. The conduit of this Environmental pollution is through the soils/sediments, water and air. Most environmental pollution effects appear initially latent with imminent harmful effect at long exposure. Two major sources of pollution [17] contributions are through human (anthropogenic) and nature (biogenic) activities.

Various definitions of pollution exist depending on ones applicative use. Pollution is the release of chemical, physical, biological or radioactive contaminants to the environment or the introduction into soil, water, and air of foreign materials, micro-organisms, chemicals, toxic substances, wastes, or waste water in a concentration that makes the medium unfit for its next

intended use. It also applies to surfaces of objects, buildings, and various household and agricultural products.

The widely used definition of pollution is " the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structures or amenity, or interference with legitimate uses of the environment" [18].

Sometimes, the words, pollutants and contaminants are interchangeably used, but distinction exists between the two in their narrow definitions. A contaminant is a substance present in the environment due to human activity that will not cause any noticeable harm. Contaminants exist in the environment as substances that exert harmful effects in the form they enter the environment. A substance when present in greater than natural concentration as a result of human activity such that it has a net detrimental effect upon its environment or upon something of value in the environment is regarded a pollutant [19, 20]. Primary pollutants cause more while secondary pollutants cause less harmful effects. A distinction exists between primary and secondary pollutants. Primary pollutants impact directly from the source of emission, while secondary pollutants are produced by the transformation and by chemical and physical reactions that primary pollutants are subjected to in the atmosphere e.g. photochemical and acidification. For instance, ozone is a secondary pollutant which reaches the highest value in areas distant from the emission sources.

Volatile Organic Compounds (VOCs) is a group of pollutants which constitute to a larger extent, grave environmental havoc as their presence constitute grave environmental heath effects. Strictly speaking, Volatile Organic Compounds (VOCs) contribute immensely to environmental pollution in all natural and man-made activities.

VOCs from the air and water are finally leached into the soil, thereby polluting the agricultural products and soil fauna as their final destination. They are important class of environmental pollutants that are uniquely found in the soil, water and air mostly in urban, agricultural and industrial centres. The term VOCs also refers to those groups of organic compounds which are present in the environment as gases, liquids and solids. These include carbon containing

compounds excluding elemental carbon, carbon (II) oxide and carbon (IV) oxide. They also encompass non-methane volatile organic compounds (NMVOCs) and reactive organic gases (ROGs).

Others include:

- Hydrocarbons (HCs) e.g. ethane (methane, ethane, octane etc); ethene and ethyne.
- Aromatic Hydrocarbons (AHs) e.g. Benzene, Toluene, Ethyl benzene, xylene (BTEX).
- Polyaromatic Hydrocarbons (PAHs) e.g. Naphthalene, Anthracene, Phenanthrene, Benzo (a) pyrene etc.
- Polychlorobiphenyls (PCBs) e.g. Chlorinated, Oxygenated, Sulphur and Nitrogen containing organic compounds.

The emergence of great factories and burning of enormous fuels and coal that resulted in unprecedented air pollution and excessive discharge of chemicals added to the growing load of untreated human waste.

It now becomes absolutely necessary to protect mankind from excessive contamination due to petroleum spills by developing quantitative, valid analytical methods to assess and quantitate the contaminants in soils.

1.5.1 Sources of Contamination

Every environmental pollution originates from a source. The source is particularly important, because it is the logical place to eliminate pollution.

VOCs and other petroleum contaminants employ many routes to infiltrate the environment. The sources can be categorized as:

(i) Anthropogenic- due to human activities.

(ii) Biogenic – evolving from natural events [17, 19, 20].

They are present in the environment mainly from human activities which include:

- Petroleum spills, leakage, distribution and petrochemicals storage.
- Motor vehicle emissions from exhaust
- Breathing air at Gasoline stations [7]
- Natural gas flares and explosions

- Combustion/burning of fossil fuels e.g. power stations
- Industrial processes such as food manufacturing etc
- Industrial/domestic solvent/chemical usage e.g. cleaning fluids, used motor oil etc
- Nuclear waste disposal and land filled wastes.
- Agricultural activities [21].

Each source is specific in releasing peculiar types of pollutants while a source may produce more contaminants at the same time.

Contributions from natural or biogenic sources to environmental pollution are significant in recent times and these include:

- Emissions from plants and trees
- Natural forest fires and biological decays.
- Release from Volcanic eruptions, lightning strikes, oceans and sand storms.
- Activities of wild animals
- Anaerobic processes in bogs and marshes [22, 23]

1.5.2 Concern

VOCs and other petroleum contaminants do not decompose nor breakdown easily and could remain in the soil, sediment, and water for a long time.
VOCs from crude oil spillages and leakages could cause huge damage to natural resources, human health, terrestrial and marine life [24]. They play vital role in contributing to the environmental problems as indicated by:

- Ozone depletion in the stratosphere
- Global warming and greenhouse effect
- Accumulation and persistence in the environment
- Photochemical formation of ozone [21].

1.5.3 Effects on Human Health

VOCs and some liquid petroleum hydrocarbons exposure may cause short or long term health effects depending on the dose and type of pollutants,

exposure time and route, individual's constitution, age and sex. Short term effects on human include:

- Headaches, dizziness, fatigue and nausea
- Sore throat inflammation
- Chest pain and congestion
- Lung and skin tumours
- Eye and skin irritation and rashes from oil spills [18]
- Haemolytic anaemia.
- Affect enzymes producing red blood cells [25].
- Allergies and immune diseases

Long term exposure may lead to:

- Peripheral neuropathy (numbness in the feet and legs) [7]
- Cancer
- Asthma
- Kidney and liver damage

Long term exposure may finally lead to

- Harm to the brain and nervous system
- Foetal damage and decreasing fertility
- Increased incidence of death [18]

1.5.4 Environmental Fate:

Living in an area near a spill or leak of petroleum products and touching soil contaminated with such will definitely expose someone to the risk. The risk of atmospheric pollution by ozone [26, 27] is high on children, young people and adults as these spend more time in the open air.

Migration of VOCs through the soil tend could result in low as well as high concentrations depending on the type and location of soil. Chemical and physical characteristics of the soil system have influence on the transformation, retention and movement of pollutants through the soil. [27, 28].

1.6 Aims of the Research

The aim of this work was to study, develop and implement validated and traceable methodology for qualitative and quantitative assessment of petroleum contaminants in soils of Niger Delta under Tropical weather conditions. The method involved the use of a Gas Chromatograph fitted with FID capable of split injection with Varian CP-Sil-GC capillary column and Combi Pal auto-sampler.

The ultimate goal was to achieve the following objectives:

- Developed an optimized GC-FID method for the analysis of petroleum hydrocarbons and key biomarkers of crude oil contamination in soil such as benzene, toluene, ethylbenzene and xylene (BTEX). A profile of these compounds and several Liquid Hydrocarbons was established.
- Evaluate the concentration range of these contaminants using capillary gas chromatographic method to identify and quantify them.
- Assess the penetration and migration levels of the contaminants within the sampling area.
- Compare the levels of hydrocarbon contamination at different depths at each location.
- Collate the contaminants into groups by their characteristic chemical similarities using Chemometric Clustering of Observations.
- Recommend remediation and possible preventive actions to the oil industries, state and local Governments based on the result of the analysis.

The preliminary study in chapter 3 involved the use of GC-FID to develop a method to investigate the retention and absorption profiles of the following aromatic hydrocarbons – benzene, toluene, ethylbenzene, total xylenes and a few hydrocarbons.

This study was limited to the sites located at Ikot Ada Udo in Akwa Ibom State, South-South of Niger Delta, Nigeria. All soil samples were collected from these sites.

1.7 Design of Experimental work

The entire process of development of method for the analysis of the petroleum contaminated soil samples involves the following principal stages:(1) Sample Acquisition (2) Sample Storage (3) Sample Stabilization (4) Sample Preparation (5) Separation Technique (6) Detection (7) Validation.

However, the implementation of the analysis plan was outlined in a schematic format in Figure 1.5 in order to depict the major routes earmarked for the operation from sampling point on site to analysis in the laboratory. The proposed procedures were necessary because the chosen sampling site was distant from the analysis point.

Three planning routes were suggested and decision was made on the most favourable, practically vulnerable and easy to operate route.

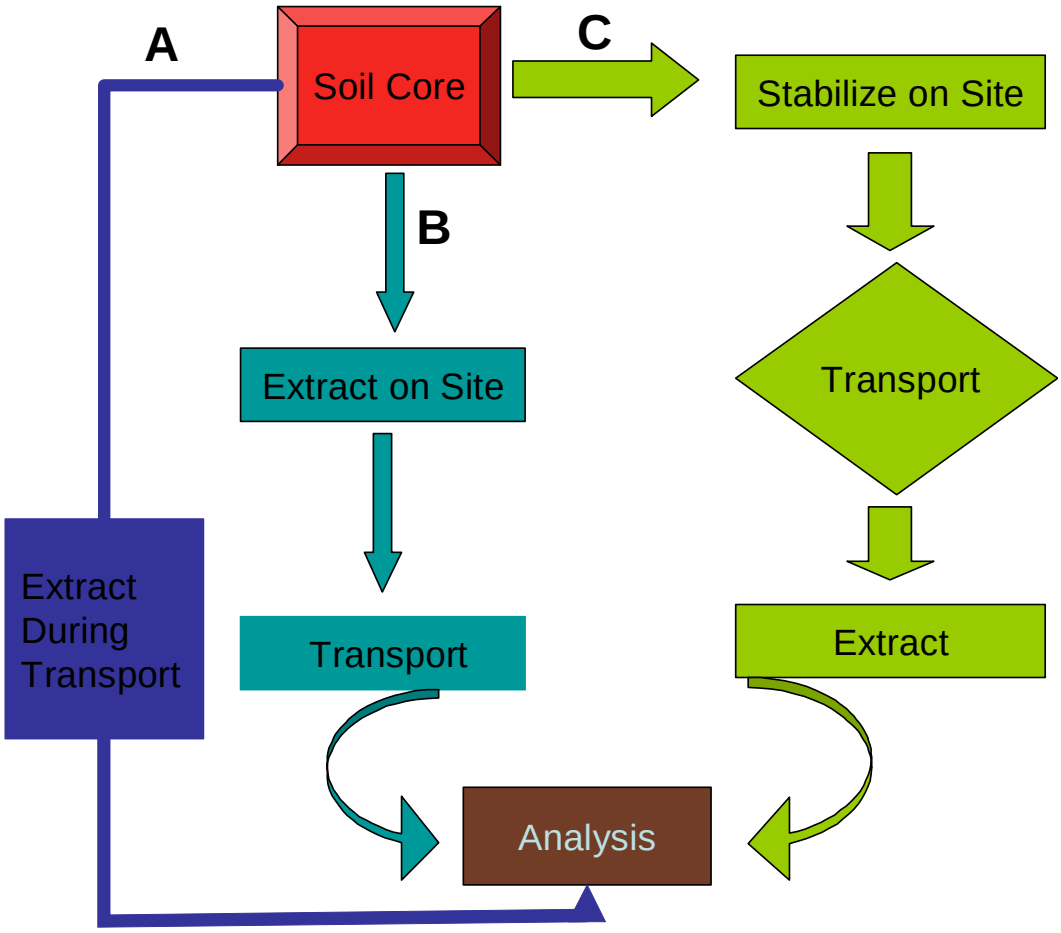

Figure 1.5 Planning Routes for sampling from the site to the Laboratory.
Route A suggested extraction of the contaminants using silicone patch in a vial during transportation immediately after sample collection.
Route B needed sample extraction carried out on site and transported to the laboratory for analysis.
Route C demanded the sample stabilized on site and transported to the laboratory for extraction and analysis.

Best engineering and analytical judgment was applied to choose route C which eventually formed the basis by which this work was carried out.

Gas chromatography (GC) still remains the most important single technique for oil spill identification partly because the equipment is relatively cheap and readily available, easy to operate with small amount of operator time and considerable amount of information can be gathered on using a high resolution (capillary) column. A method based on the soil extraction of the samples prior

to analysis using GC-FID provides a good analytical tool to determine the petroleum contaminants.

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CHAPTER 2

BACKGROUND

2.1 Introduction

Gas chromatograph (GC), developed decades ago for the separation of substances has not lost it prominence, and remains the appropriate and sensitive separation technique. Gas chromatography (more properly called gas-liquid chromatography) is the separation technique of choice for organic compounds, which can be volatilized without being decomposed or chemically rearranged. The technique has been established for the determination of hydrocarbons. There have been many developments in chromatographic analysis in recent years and combined gas chromatography methods are generally preferred for the analysis of complex hydrocarbons. However, different analytical techniques have been employed in the analysis of organic contaminants as well as trace element contents of petroleum contaminated soils and crude oils. Large-scale crude oil spills are the most obvious source of hydrocarbons pollution [1, 2, 3, 4]. The use of hyphenated techniques involving hybrid of GC-FID, mass spectrometry (MS) such as GC- MS, GC- ICP, ICP-MS and GC-LC- MS proved to be the most versatile, suitable and powerful tools to undertake both quantitative and qualitative characterization of petroleum contaminants and trace elements in soil and water extracts.

Miguel [5], Akinlua et al [6], used GC coupled with ICP and related techniques to determine and characterize organic pollutants and rare earth elements in contaminated soils in Niger Delta crude oils. Replicate digested oil samples were analyzed using ICP-MS. Agadi and Al Swaidan [7] used ICP-MS to determine

vanadium in crude oil. A critical overview of the application of other analytical techniques for the analysis of trace element content in crude oil contaminated soils had been carried out [8]. Application of comprehensive two-dimensional gas chromatography [9] was also adopted for the assessment of oil-contaminated soils. GC- MS, [10] was shown to be a well-established approach for the analysis of volatile organic compounds in samples.

GC-FID methods for the determination of petroleum hydrocarbons in soil have been developed based on modifications of reported methods [11, 12, and 13]. It involves extraction of the petroleum contaminants from a known weight dry oil-spilled soil with a suitable solvent using Soxhlet Extraction Apparatus (SEA). The analysis was carried out with GC fitted with FID. The choice of GC-FID over Photo Ionisation Detector (PID) includes the following advantages which include that: (i) FID can cope with high humidity and equally handle very wet samples than PID. (ii) In FID, the flame is capable of ionising large range of volatile organic and petroleum Hydrocarbons than PID, thereby detecting a wide range of Hydrocarbons. (iii). FID is very useful in detecting lower concentrations volatile organics because of its lower detection limits (ppm levels). FID limitations [14] include ability to detect volatile hydrocarbons from non-petroleum matter and organic material such as methane and peat.

Many analytical techniques are applicable to petroleum hydrocarbons. The emphasis is on reproducible, correct and meaningful interpretation of the data obtained from these techniques. This interpretation among other things could depend on adequate knowledge and appreciation of the following:

(i) Nature and complex properties of petroleum and it products.

(ii) Vast differences between pure petroleum (crude oil) and that spilled on soils.

(iii) Partitioning characteristics of constituents in soil strata.

(iv) Extraction conditions and other peculiar situations [15].

2.2 Soils

Soil exist in many forms and its definitions are various according to application. The soil scientists identified it as the unconsolidated mineral material on the immediate surface of the earth that has been subjected to and influenced by genetic and environmental factors. On the other hand, the Engineer defines soil as the mass of unconsolidated mantle of weathered rock and loose material lying above the above solid rock. [16].

The definition of Soil [17] has it as unconsolidated earth material composing the superficial geologic strata consisting of clay, silt, sand, and gravel size particles as classified by the U.S soil Conservation Science. Soil according to [18] is "the layer of minerals and organic matter, in thickness from centimetres to a metre, on the land surface. Its components are rock, mineral and organic matter, water, and air". Soils differ in the ratio of these components and hence mechanical properties are largely determined by particle size and strongly influence the behaviour of polluting agents like hydrocarbons. The type of soil, its characteristics and behaviour towards different pollutants contribute to its future preservation and composition. Soil porosity and permeability determine the ease of flow of water through it (refer to figure 2.1).

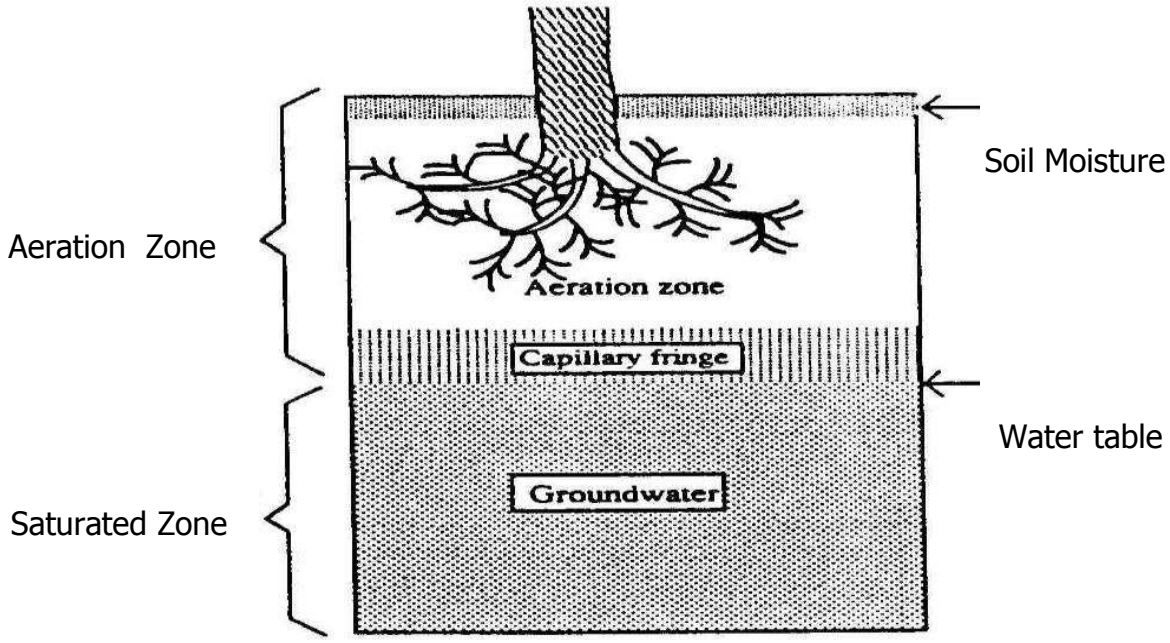


Figure 2.1 Soil nature and distribution of water underground [19].

The general region in which water is held is called the aeration zone and the water present in it is called vadose water. At lower depths, adequate amounts of water will fill voids to produce a zone of saturation, the upper level of which is the water table. Water in the zone of saturation is called groundwater. The surface tension of water is drawn slightly above the water table to a region known as the capillary fringe [19].

Finally, the contaminants will carve out their pathway by leaching into the soil, come in contact with the ground water from the water table and subsequently discharge its content into the canals, lakes, rivers and then to the sea.

Soil is not just a simple environment, lots of chemical, physical, biological, and geological factors constantly interact to vary its composition.

2.3 Contaminated Soils and Water in Niger Delta.

It is obvious that petroleum exploration and refinery activities have contributed largely to the economic growth of Nigeria but over the past decades, the Niger Delta has suffered serious human health risk and ecosystem depletion as result of petroleum spills, petroleum product leakages and other effluents generated from crude oil exploration. Petroleum products have been identified to be one of the several soil and water contaminants in this region. Petroleum and petroleum products have been the most commonly used chemicals in Niger Delta and the Diaspora. The soil and water are the main environmental media which act as chemical reservoirs for trapping and storing the contaminants released from biogenic, teratogenic and anthropogenic activities of the petroleum sector. However, the anthropogenic contribution usually outweighs the inputs from other sources. Teratogenic source include chemical, ionization or virus that alters or interrupts normal development or malformation of embryo or foetus at birth. Undoubtedly, soils are good environmental compartments to record the distribution patterns of these contaminants as they penetrate the depth of the soil. These contaminants finally reach the human populations and ultimately cause potential human effects.

Contaminated land (soil), by no means, is never identified by a layman's perception except the level of pollution is outstanding, like in cases of oil

spillages and pipe leakages. A question of what is a contaminated soil may be difficult to answer unless a clear distinction is established between contamination and pollution. Contamination was recognized as introduction or presence of alien substances or energy liable to cause damage or harm. Pollution was defined as introduction of substances or energy liable to cause hazards to human health, living resources and ecological systems, damage to structures and amenity, or interferences with legitimate uses of the environment [20, 21]. However, to distinguish between contaminated and polluted land will mean that screenings and risk assessments needed to be carried out to establish if the hazard created could result in probable harm. Sediment and soil form the major conduits by which these contaminants reside. Sediment is a soil, sand, and minerals particles washed from land into water, usually after rain and deposited in reservoirs, rivers and harbours, destroying fish and wildlife habitat, and clouding the water so that sunlight cannot reach aquatic plants. [22 and 18] described sediment as unconsolidated material transported by water surface or deposited under it with different textures, ranging from thin dust to thick gravel. The composition and characteristics of sediments will influence the pollutant content. Careless farming, mining, and building activities will expose sediment materials, allowing them to wash off the land after rainfall.

The frequency, magnitude and seriousness of environmental health problems caused by petroleum contamination in Niger Delta need urgent validation and remediation attention. Therefore, the major task facing the Niger Delta region lies on identification, assessment, evaluation and remediation of the petroleum-contaminated sites and a follow-up of the health-related actions and observations. It is clear from the analytical point of view that the quality of risk assessment and the chances for a successful soil remediation depends on the strength of characterization [9, 23]. Soils are very complex and potentially static environmental media. This presents a more basic problem of how to accurately measure the amount of contaminants in the soil. It has long been recognized that soil sampling and analysis presents a host of difficult technical obstacles

that confound attempts to quantify the concentration of various soil contaminants [24, 25].

Soils, by its condensed nature, possess some important factors that can interfere with accurate analysis which includes but not limited to:

- Matrix effects – created by other organic non-target soil components.
- Volatility of some of the contaminants (some escape during sampling, handling, storage and analytical processes.
- Chemical characteristics of these contaminants often make it difficult to bring them into solution in a fast, simple and quantitative manner [26].
- Lack of adequate analytical methods specifically developed to characterize petroleum soil contamination [24].

The public health risk associated with constant exposure to these types of contaminants will call for evaluated data and information on:

a) Types of contaminants in the environmental media.

b) Detected levels of concentration of the contaminants.

c) Location and migration of these contaminants with respect to the human populations.

d) Effect on the ecosystem, human and plant lives.

e) Preventive measures and remediation.

Therefore, protection of human health is an essential requirement of both treatment and reuse of petroleum-contaminated soils.

2.4 Health and Agricultural Risks

Petroleum-well spillages (figure 2.3 a and b), pipe line and storage (surface and underground) tank leakages, vapour emissions, gas flares, wastewater and

similar discharges associated with petroleum contamination instigates environmental health risk and agricultural defects [27, 28].
In recent decades, petroleum and petroleum products have attained the acme of its usage. Extensive amounts of these products are usually stored underground or on the surface tanks or transported in tanks and underground pipelines. Accidental discharges or spills, during transportation, land disposal facilities, engines, large generating plants and illegal dumping or ‘’bunkering” are another source of introduction of petroleum contaminants into the soils, sediments and water in South of Niger Delta.

Brady and Weil [29] in their epidemiological studies reported that the number of deaths resulting from these contaminants equal to or exceed the number of deaths caused by road accidents. Varying amounts of chemical pollutants and contaminants such as sulphur, mercury, nitrogen oxides, and vanadium is also known to contain Pet-coke- a solid residual carbonaceous product (coal), used as fuel [9]. Accumulation of metal pollutants from the leaves and roots of higher plants have been reported [30]. Elemental deposition on plant materials was studied [31, 32, 33]. Reports show that some trace elements are likely source of environmental pollution; causing catalytic poisoning and corrosion of refining columns and turbines [6, 34].

The fate and integrity of soil, sediments and waters continue to degrade due contaminants accumulated from crude oil, petrochemicals and petroleum related products. From the environmental point of view, the parameter to characterize the soil and sediment will depend on organic contaminants and the metals, especially the heavy metals content and their species in the soil [5].

Figure 2. 2 **Effect of Petroleum spillage in south Niger Delta village farm. (a) Cassava and other staple crops in the area were massively killed and ultimately left to decay. (b) Women and farm owners mourned their lost.**

Problems of health risks and high capital investments are imminent when considering the use of these soils for agricultural, industrial and urban activities.

2.5. Soil Extraction Techniques

Irrespective of the methods chosen to analyze a soil, sample preparation may be the precursor, where the components of interest are extracted from the sample matrix and concentrated for analysis. Sample preparation in contrast to current advanced instruments is dependent on long established conventional and classical techniques. Aside from taking a descriptive view of the various

techniques, their advantages and limitations, it should be noted that not every technique is specific to purpose and not one suffices for all applications.

2.5.1 Liquid-Liquid Extraction (LLE) and Solvent Extraction

Liquid-liquid Extraction (LLE) or Solvent Extraction is an analytical method that separates two practically immiscible liquids (usually water and organic solvent) by extracting one substance from the liquid phase into another liquid phase using separating funnels. Suppose an analyte (solute), **S**, is partitioned between two phases 1 and 2, the ratio of the concentrations of **S** in the two phases will be constant.

The Partition or distribution coefficient, K, is the equilibrium constant for the reaction:

S(in phase 1) $\rightleftharpoons$ **S** (in phase2) **2.1**

$$K = \frac{[S_1]}{[S_2]} \quad \text{..... 3.2}$$

Where, S1 = organic solvent and S2 = solvent 2 e.g. water.

The method has been shown to be a cost efficient and effective process in treating soils, sediments and sludge containing primary organic (VOCs) contaminants, PCBs, petroleum wastes, and for spectrophotometric determination. The process has been effective in removing organic contaminants from paint, coal tar, pesticide/insecticide, oil, synthetic rubber process wastes, and drilling mud. Some of the limitations include:

- Extensive pre-treatment of the waste to remove or break up large clumps.
- Reduces the amount of contaminant extracted if strong acids and bases, detergents are present in the waste.

- The presence of lead and other inorganics may interfere with the removal of organic materials.
- Uses much solvents
- Takes longer time
- Exposes user to hazard from solvent
- The final products still needs further clean- up and concentration before being used in the GC [35, 36, 37].

Final products still need further clean-up and concentration before being used in the GC. [35, 36, 37].

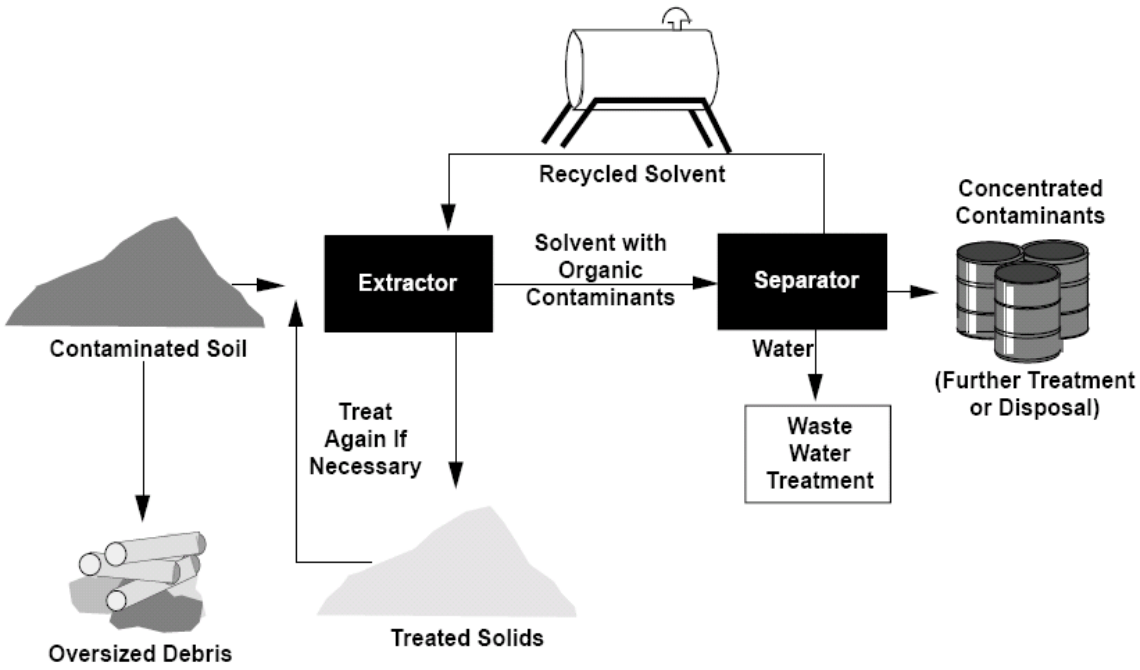


Figure 2.3 Solvent Extraction Process

Conventional (LLE) extraction processes involve several manipulative steps thereby becoming labour intensive, time consuming as Figure 2.3 illustrates.

In attempts to improve the conventional solvent extraction method, other techniques were developed principally to optimize extraction efficiency using less time and minimal solvent. A traditional and classic Soxhlet extraction procedure falls under solvent extraction. It has many applications and specific extraction capacities though with inevitable limitations still proved useful where

other new technique may be ineffective. Soxhlet extraction is a purification technique developed to extract compounds that have limited solubility in a solvent and the impurity is not soluble in that solvent and vice versa. Normally the desired solid material is placed inside a dry, porous paper thimble which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is equipped with a condenser and the solvent heated to reflux. The solvent vapour rises through the extraction thimble and floods the chamber housing the sample and the thimble. The condenser ensures that any solvent vapour cools and drips back down into the chamber which slowly fills with warm solvent. The chamber is automatically emptied by a siphon side arm when almost full with the solvent running back down to the distillation flask (refer to section 4.3.1). The purified compound is then extracted from the solvent using a rotary evaporator while the impurities stay in the thimble. The extraction cycle may be allowed to repeat as desired.

2.5.2 Supercritical Fluid Extraction (SFE)

Supercritical Fluid Extraction (SFE) [38] was introduced as an extraction technique based on specific properties of a solvent above the critical point [42]. A supercritical Fluid (SCF) is a compound that exists at temperature and pressure above the thermodynamic critical point. Pure gaseous components of this compound cannot be liquefied above its critical temperature regardless of the applied pressure. The critical pressure is the vapour pressure of the gas at the critical temperature. However, in the supercritical environment, only one phase exist- the fluid, a term applied to neither gas nor liquid with the fluid density as a strong function of temperature and pressure . Supercritical Fluid Extraction uses CO_2 under extremely high pressure to achieve separation of solution.

The basic principle of SFE is that when the feed material comes in contact with supercritical fluid (SCF) than the volatile substances, it partitions into the supercritical phase. After dissolution of soluble material, the SCF containing the dissolved substances is removed from the feed material. The extracted

component is completely separated from the SCF at high temperature and pressure. The SCF is then recompressed and recycled. The advantages of this method include:

- Extracting analytes faster.
- Inexpensive and contaminant free
- High boiling components are extracted at relatively low pressure
- Dissolving power of the SCF is controlled by pressure and or temperature
- SCF is easily recoverable from the extract due to its volatility
- Thermally labile compounds can be extracted with minimal damage as low temperature can be employed by the extraction
- Separations not possible by more traditional processes can sometimes be effected
- Modifiers (e. g methanol to CO_2) can be added to the SCF to change its polarity for more selective separation. However, these disadvantages exist:
- Elevated pressure required
- High capital investment for equipment [39, 40] is the demerit.

SFE technology appears to be the most studied and applied application in textiles, pharmaceuticals, analysis of VOCs, food, vitamin extraction and decaffeination of coffee [41, 42].

2.5.3 Microwave Assisted Extraction (MAE)

Microwave-Assisted Extraction (MAE) was developed [43] for fast, efficient and unconventional extraction of analytes from solid matrixes. In order to avoid interferences with radio communications, microwaves (electromagnetic radiations) domestically and industrially are operated at 2.45 GHz though it has a frequency range of 0.3 to 300 GHz. Owing to their electromagnetic nature, microwaves possess electric and magnetic fields which are perpendicular to each other. The electric field causes heating via two simulation mechanisms referred to as dipolar rotation and ionic conduction, thereby producingoscillations which in turn generate collisions with the molecules available in the surrounding and give off thermal energy into the medium.

Microwaves unlike the conventional conductive heating swiftly heat the whole sample simultaneously [43, 44].

2.5.4 Accelerated Solvent Extraction (ASE)

Accelerated Solvent Extraction (ASE) is another new solvent extraction technique developed to provide faster and rapid extraction time coupled with drastic solvent reduction. ASE operates at high temperatures and pressures to achieve extraction using the traditional organic solvents and without changing the state of the liquid solvent. ASE has been employed in the sample extraction for Semivolatile organic and persistent organic pollutants (POPs) in soils [45, 46], PAHs in smoked fish [47]. Recoveries were compared with Soxhlet extraction and sonication and have been shown to be exceptionally effective extraction technique when compared with MAE, Ultrasonic Extraction (USE), steam distillation and SFE. Apart from reduced solvent and time of extraction, ASE extraction protocols are simple with low pre-extraction cost [45].

However, quantitative extraction comparison was made for selectivity, recovery and extract quality on Soxhlet Extraction (SE), Supercritical Fluid Extraction (SFE), Pressurized Liquid Extraction (PLE), Subcritical Water Extraction (SWE) for environmental solids [48].

2.5.5 Static Headspace Analysis (HS)

The Static headspace technique often made use of heat at a specified time to drive the analyte to attain equilibrium between the sample matrix and the gas phase.

The technique provides a convenient means of sampling volatile analytes for GC analysis. The technique is used to concentrate and carry out further analysis of VOCs.

The technique can be compared with purge and trap in sensitivity, providing fastest and cleanest method for VOCs analysis. A headspace is created (refer to figure 2.4) when a sample, (dilution solvent and matrix modifier) in a sealed vial is equilibrated at a set temperature for a particular time. A portion of the vapour in the headspace is taken out by a syringed needle to GC system for

separation with particular care to sample preparation and proper instrument adjustment.

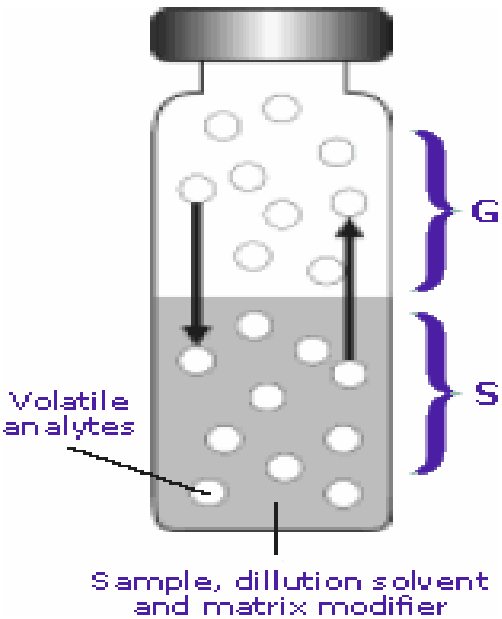


Figure 2. 4 Headspace vial. [49]
G= gas phase,
S= sample phase

A state of equilibrium is established in the headspace, the moment the sample is introduced into the vial and sealed and volatile components begins to diffuse into the gas phase.

The equilibrium distribution of the analyte between the sample phase and the gas phase is given by:

$$K = \frac{[C]g}{[C]s} \quad \text{.....} \quad \textbf{2.3}$$

Where Cs = Concentration of analyte in sample phase.

Cg = Concentration of analyte in gas phase.

It is established that compounds with high K value distribute more readily than those with low K, and do so with high sensitivity and low detection limit. Accuracy of result depends on careful and adequate sample preparation, which will in turn;

- minimize possible contamination

- maximize the concentration of the desired volatile analyte in the headspace.

It is noted that the concentration of the analyte depends on a factor called **Phase ratio, β**, defined as:

$$\beta = \frac{[V]_g}{[V]_s} \quad \text{..... 2.4}$$

Where, Vg = Volume of gas phase (Headspace)

Vs = Volume of sample in vial.

The final concentration of the analyte in the headspace is calculated from the ratio obtained from K & β, viz;

$$\beta = \frac{[C]_o}{[K + \beta]} \quad \text{..... 2.5}$$

Where Cg = Concentration of analyte in the gas phase.

Co = Original concentration of the analyte in the sample [49, 50]

2.5.6 When to Use Headspace

When direct injection is not possible especially if results are better achieved than with direct injection.

It is useful when samples need previous extraction e. g alcohols in blood, water pollutants and if volatile compounds only are analyzed.

2.5.7 Advantages and Limitation

It has good detection limit, precision and accuracy and is suitable for small samples. The ability to eliminate matrix effect and sample preparation is not needful

No volatile compounds are introduced into the injector or the column [51].

The limitations of the technique include the following:

- Needs no internal standard for quantification
- Quantification is only done in the headspace
- Require special instrument.

2.6 Dynamic Headspace - Purge and Trap

This is a technique in which the volatile analyte is quantitatively extracted from the sample matrix by passing gas continuously to the sample.

Purge and Trap is a method employed to remove liquid or solid samples, (e.g. water and soil), concentrate the analytes before introducing them into the GC instrument. It is actually a form of thermal desorption technique.

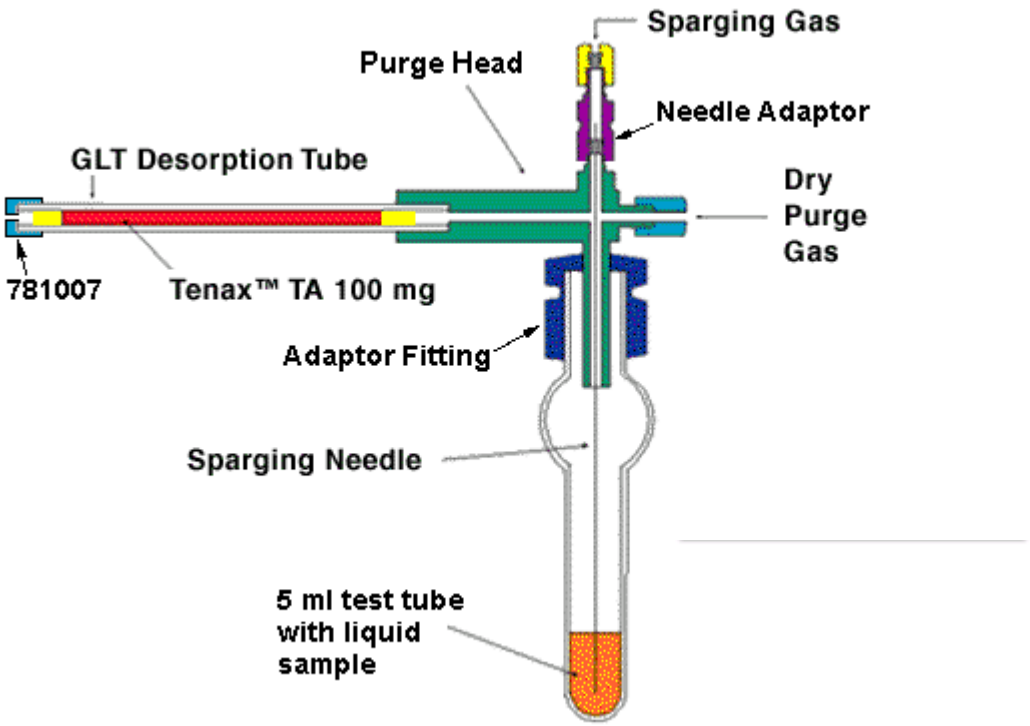


Figure 2.5 Purge and trap (Scientific Instrument Services Inc. [52]).

Purge gas (helium) is bubbled through the sample in a vial maintained at a known temperature. The sample volatiles are then passed through an adsorbent tube containing suitable adsorbent compound e.g. Tenax TA as shown in Fig.2.5. The trapped volatiles at the adsorbent tube is then introduced to the GC for desorption and analysis.

2.6.1 Advantages and Limitations

- High sensitivity [35, 36]

- Recommended for VOCs analysis [53]
- Can remove 100% analyte from sample [36]
- Quite suitable for heterogeneous samples

Its application had been employed in rapid differentiation studies of crude oil in polluted soils samples [54], rapid detection of hydrocarbon in polluted soil [55], diagnosis of used engine oil [56] and was shown to be sensitive in the analysis of volatile fragrance and flavour compounds [57].

The demerits of this procedure possibly are the introduction of volatile contaminants into the system and no adequate internal standard.

2.7 Solid Phase Microextraction (SPME)

Pawliszyn's Research Group, of The University of Waterloo, developed SPME technique in the late 1980s [58] to bring an innovative, fast and solvent free technology into chromatographic analysis of samples (liquid and gas).

Solid Phase Microextraction, SPME, is a relatively new and versatile sample extraction technique adopted for the analysis of volatile or semivolatile organic environmental pollutants, fragrance components, flavours, and other liquid and solid samples [58, 55].

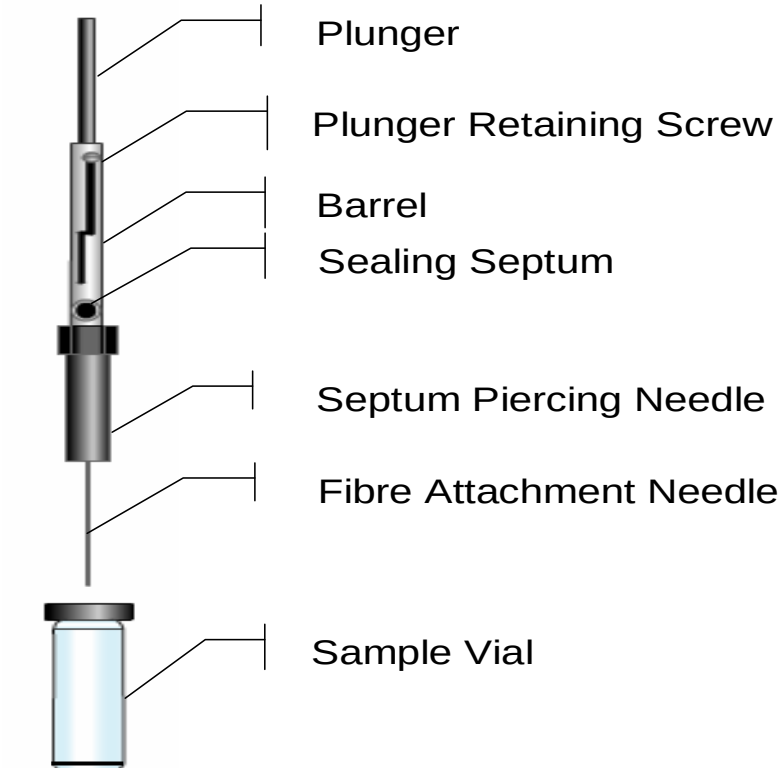


Figure 2.6 Headspace (SPME) apparatus

Its application to soil analysis is yet quite limited, but SPME technique offers unique capacities to chromatographic analysis of some samples in different matrices [59].

SPME is an inexpensive and simple technique made up of fused silica fibre (1 cm long) that is coated with a polymeric phase. This fibre is encased in a length of stainless steel tubing to give it the rigidity and the required mechanical strength for routine analysis (figure 2.6). The stainless tubing bearing the SPME fibre is contained in a syringe specifically designed for this sampling method [60, 61].

Solid Phase Microextraction sample preparation works in two stages namely, extraction step and the desorption step. Essentially, the extraction step (figure 2.7) involves extracting solutes from sample into the SPME adsorptive film-coated fibre by the piecing of the septum of the sample vial by the needle to expose the coated fibre inside the syringe into the headspace or the liquid medium. Partition of the analytes occurs between the sample and the coated fibre at equilibrium.

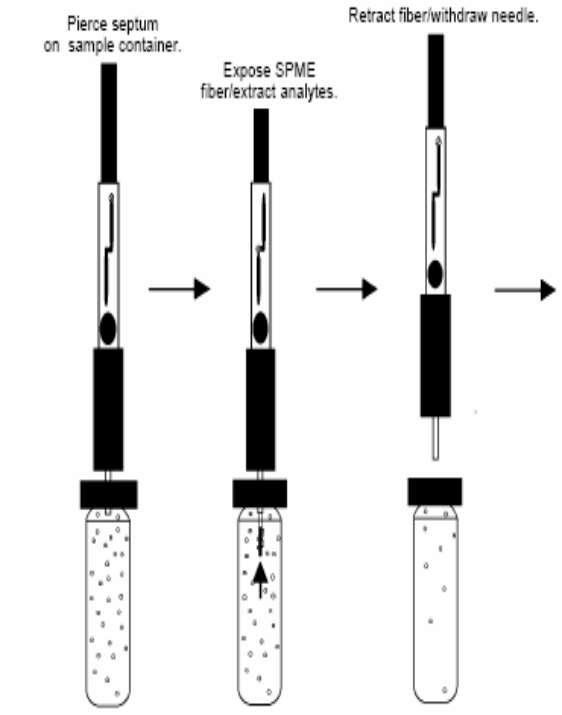


Figure 2.7 Extraction stages [61]

The fibre is then withdrawn into the needle through the septum after the equilibrium state. The absorbed analytes are then desorbed (figure 2.8) from the SPME into the GC mobile phase through the injection port. In this process, the fibre is exposed to release or desorp the analytes into the GC column.
The equilibria are established between the concentrations of the analyte in the sample and the polymer coating on the fused silica fibre.

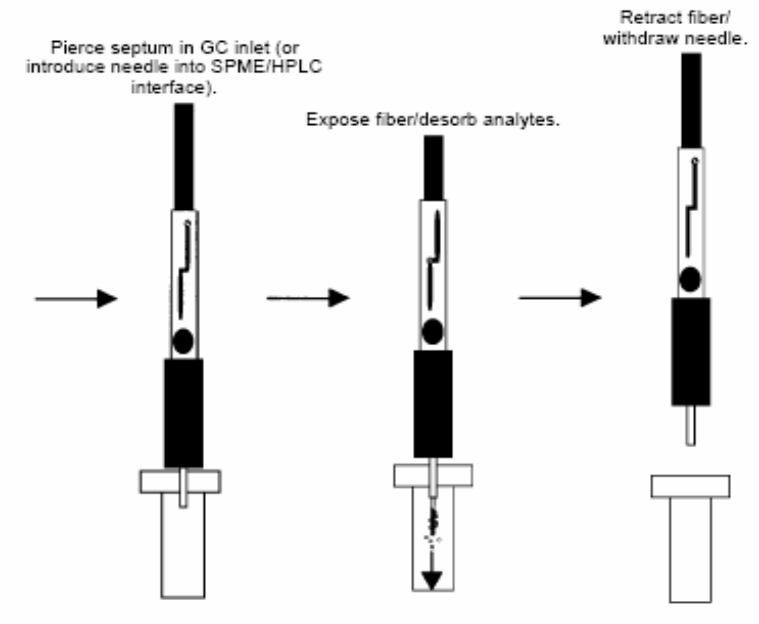


Figure 2.8 Desorption stages [61]

The amount of the analyte adsorbed by the coating at equilibrium is directly related to the concentration of the analyte in the sample [37].

$$n = \frac{K_{fs} V_f C_c V_s}{K_{fs} V_f C_c + V_s} \quad \text{.....} \quad \textbf{2.6}$$

Where n = mass of analyte
Cc = initial concentration of analyte

K_{fs} = partition coefficient for analyte and sample

V_f = Volume of coating

V_s = Volume of sample

2.7.1 Advantages and limitations

The technique offers good sensitivity and solvent free analysis. It is simple, fast and minimizes sample preparation and disposal of toxic solvents [59]. SPME handles difficult samples [61] hence cost effective with high RSD. It is not reproducible. A considerable amount of literature has been published on the efficacy of the various extraction techniques of VOCs and other organics. Speed, reduced use of solvent and labour lost form the crux of the findings. However, those accounts overlooked the efficiency and recovery of petroleum or crude oil contaminants from spilled soils. Higher recoveries and good extraction efficiency is consistent with Soxhlet extraction because the refluxing solvent repeatedly washes reasonable weight of the sample thereby extracting the desired compound (analytes) into the extraction vessel. The recovery of liquid hydrocarbon contaminants is dependent on the extraction technique such as the traditional Soxhlet. The conventional and classic Soxhlet extraction method has been consistently used as a reference and comparative parameter to all other techniques.

In the sample preparatory stage of this work, Soxhlet was adopted in extraction of the petroleum hydrocarbons from the crude oil contaminated soil sampled three months after spillage from tropical region of Niger Delta, Nigeria. The efficiency of a Soxhlet extraction depends on the choice of solvent as well as optimized extraction time. SEA is readily and easily assembled, inexpensive and accessible to every laboratory. Soxhlet is still a preferred method for the extraction of crude oil from soil with high yield of extracts than SFE, PFE, ASE and MAE [42]. However, this extraction technique has a number of limitations includes the use of solvent, delayed extraction time.

2.8 Sampling Protocols

The importance of sampling to the overall processes of analysis is emphasized with quality control issues. It is worth mentioning that sampling is the first significant determining step in every analysis. Sampling is perhaps the major and persistent source of error in the analysis of organic pollutants in soil and sediments thereby presenting problems for the analyst and investigator. Experienced samplers and specialized sampling instruments are the essential precursor.

Sampling protocols are written descriptions of the detailed procedures that should be adopted during sample collection, mixing, packaging, labelling, storage, preservation, documentation and transportation [63]. However, Research was carried out to evaluate and develop new approaches aimed to improve soil and sediment sampling in order to obtain more representative subsamples that will reduce the errors that commonly occur during sample collection, mixing, treatment and handling [64]. The collection and preparation of samples form the crux of any environmental assessment programme. A sample should be a small and informative representative of the environment and therefore, the method of collection and sample size should be properly chosen. Various decisions and tasks must be addressed before a sampling plan of environmental study could function. These may include:

- Definition of study and data quality objectives (DQOs) of the study drawn up.
- Choice of target population (analytes), sample size and location
- Identification of the measurement methods
- Development of sample plans
- Exploratory study, number of representative, Quality Control, (QC), blank and samples.
- Sample location, method of sampling and need for supplementary samples.
- Cost and time of analysis
- sampling equipment and containers
- sample preservation and preparation.

- Statistical analysis of data
- Formulation of Quality Assurance parameters
- Assessment of risk hazards and safety requirements.
- Choice of control sites [63].

2.9 Sampling Plan

Sampling plans should be written with the aim of utilizing the information to check the sampling protocols [22]. A sampling plan defines the objectives of the sampling and provides specific description of the data to be collected, the interval of data collection, and the subjects from whom the data will be collected. Outlines of the various decisions that must be introduced into a sampling plan should include:

- Choosing the sampling location
- Selecting the size and number of samples
- Selecting the frequency of sampling and type of sample (composite, grab or discrete etc)
- Choice of sampling devices, equipment, containers and cleansing requirements and preservation.

Preliminary testing should be carried out because the distribution of petroleum contaminants in the soil environment can be random, stratified, systematic (uniform), patchy or following a certain gradient [22, 63].

Mason [18] asserted that effective sampling could be carried out in two phases, namely :(I) Exploratory or preliminary (surveillance) phase (II) monitoring or assessment phase.

An exploratory study provides preliminary information or history facts about the sampling site and estimates the variability and trends in the population data. The exploratory sampling gives knowledge of the chemical species of interest and their concentration variability. This step will also help to develop a sampling plan that will take advantage of the selection of appropriate sampling equipment, sampling protocol, analytical methodology and required quality control parameters with much accuracy and less cost. A preliminary study

whose statistics is accurate is informative enough for the development of data quality objectives and decisions about sampling data requirements [18, 22]
Monitoring and assessment should be designed in steps to provide information on the variation of the concentration of analyte of interest over time or given area. Monitoring stage is developed based on the results procured from the surveillance study. Preliminary sampling/screening is supposed to indicate the contamination levels in the effected area. Preliminary study should provide information about the type and behavioural pattern of the pollutants at the designated environmental site.

2.10 Sampling Theory

The purposes of sampling and the specific information that one expects should be properly linked to the design of a sampling plan.
In considering sampling design, different sampling approaches can be used depending on the selection procedure of the samples. However, depending on the complexity of the sampling sites and the objectives of the study, approaches to sampling may change. The location for collecting a soil sample, the position of the sample pit and the location of the cores was based on random, stratified, systematic and judgmental approaches. Whichever approach is used, it must provide different information to meet with the data quality objective by selecting representative samples. Representative sample simply means sample resulting from a sampling plan that can be expected to reflect adequately the properties of interest in the parent population.
Uniform fields can be sampled based on probability sampling procedure in which the population samples are selected in a simple random stratified random, cluster sampling, double sampling and systematic sampling.
Sampling patterns are selected with or not with statistical approach according to the scope of the sampling and to the need of obtaining representative samples.

In a simple random sample, n items are taken from a population of N items in such a manner that all possible combinations of n items have the same probability of being taken [65]. With a simple random system, each item is

selected separately, randomly and independently of previously drawn units (probabilistic selection of the sample). Mason [18] reported that sampling method may be subject to bias.

The purpose of designing a sampling programme is to provide the most efficient methods to reach valid and relevant conclusions from the investigation of soil, with due regard to cost or resource use commensurate with sampling programme objectives.

A stratified random sample is taken from a field that has been divided into several subunits or quadrants from which simple random cores are obtained. Stratification is defined as "the division of a population into mutually exclusive and exhaustive sub-populations (called strata), which are thought to be more homogeneous with respect to the characteristics investigated than the total population" [66].

The reason for taking stratified samples is to obtain a more representative sample than that which might otherwise be gotten through random sampling.

The whole purpose of stratification is to increase the precision of the estimates and control the sources of variation in the data.

Figure 2.9, illustrates the common soil sampling plans. In situations where there exist no adequate information to develop a conceptual model for a site or to stratify the site, it becomes more imperative to use random sampling. Equally, stratified random sampling should give increased precision if the strata are homogeneously selected than considering the entire population. More stratification should result in greater increase in precision [18].

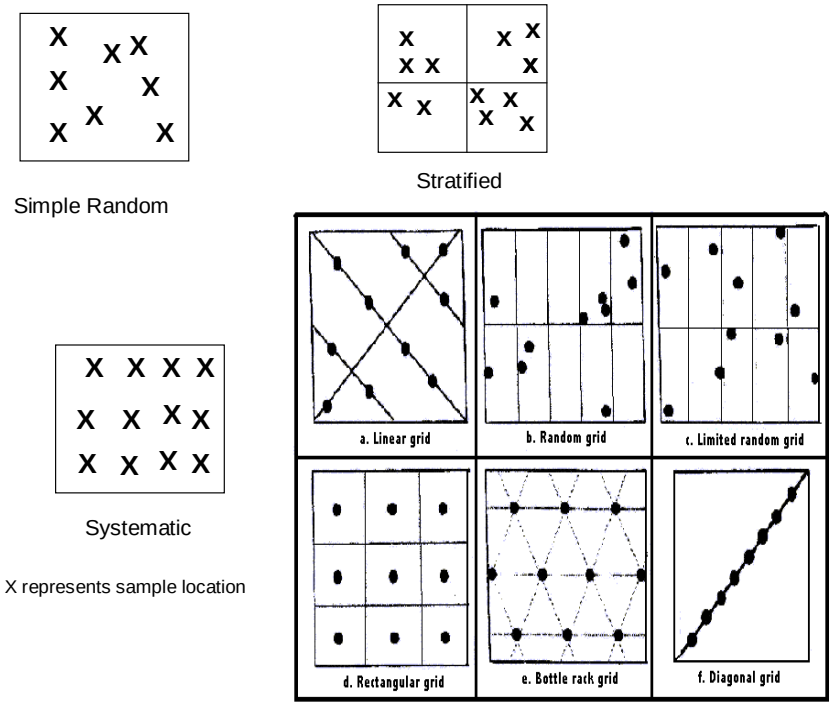


Figure 2.9 Sampling plans. x and • represent sample locations [19].

Systematic sampling plan has been widely accepted because it is straightforward and potentially increases the accuracy of soil tests.

Judgment sampling subjects population samples to selective choice of an expert. The judgment of the expert is based on a prior knowledge of the sampling site and /or on the visual site observation e. g high contaminant concentrations.

2.10.1 Number of samples

The quantity or number of samples to collect in order to achieve specified accuracy and precision is related by mathematical relationships [18] given in the equation below.

$$n \geq \left[\frac{(Z_a + Z_b)}{D}\right]^2 + 0.5Z_a^2 \quad \text{..... 2.7}$$

For a one –sided, one sample t-test and

$$n > 2\left[\frac{(Z_a + Z_b)}{D}\right]^2 + 0.5Z_a^2 \quad \text{..... 2.8}$$

for a one-sided, two sample t-test

Where n= number of samples

Z= percentile of the standard normal distribution

a= probability of a Type I error

b= probability of a Type II error

D= minimum relative detectable difference/coefficient of variance

The actual quantity of sample to collect could be worked out using the following relationships:

$$A = \frac{\left(W_1 \cdot W_2 \left(S_1^2 - S_2^2\right)\right)}{\left(W_2 - W_1\right)} \quad \text{..... 2.9}$$

$$B = S_1^2 - \left(A/W_1\right) = S_2^2 - \left(A/W_2\right) \quad \text{..... 2.10}$$

Where the optimum sample weight is given by:

$$W_{opt} = \frac{A}{B} \quad \text{..... 2.11}$$

With most environmental sampling, the weight of the sample should be at least six times the minimum weight (W_{min}) which is:

$$W_{\min} = \frac{A}{\left(X_{av} - G\right)^2} \quad \text{..... 2.12}$$

Where:

A = homogeneity constant

B = segregation constant

W_1 = weight of small random samples from a preliminary study

W_2 = weight of large random samples from a preliminary study

(At least 10 times the weight of w_1).

S_2 = Variance

Xav = average concentration in random samples

G = background concentration.

Total variance for the soil material (S_T^2) can be estimated by the following equation:

$$S_{T^2} = \frac{A}{(N * W)} + \frac{B}{N} \quad \text{..... 2.13}$$

Where:

N = number of samples

W = weight of small samples

N*W = total weight (W) of material from small samples.

2.10.2 Sampling Equipment

Soils are usually treated as two or three-dimensional materials and proper sampling tools are needed by the analyst for correct sampling. The sampling equipment used depends on the nature of analyte, the depth (surface, subsurface, trench sampling) and homogeneity of soils. Soil Samplers are chosen for purposes of sampling analyte contamination at the surface in cases of recent contamination, oil spills and small scale migration of analytes and depth sampling when the analyte is volatile and has long migration period in the soil [63, 67, 18]. These latter situations exhibit migration downward into the deeper soil layers. Volatile Organic compounds demand greater depths of sampling and specific sampling devices are required to obtain a representative sample.

Surface (subsurface) or shallow depth sampling (typically about 15-30cm) involves different sampling devices such as soil augers and tubes, stainless spoon/shovels, stainless ring sampler, little corers, trench and punch samplers, soil probes. Some of these samplers are not suitable for sampling soils

contaminated with VOCs due to their volatilization during the sample acquisition, mixing, transfer to containers and treatment. For volatile constituents, cohesive-sample soil core method may be useful. Samples for volatile organic analyses must remain as undisturbed as possible.
Sampling devices for soils and sediments must be decontaminated between successive collections to avoid contamination of the individual samples. Therefore, the sampling tools must be thoroughly cleaned prior to use.

Cleaning procedures have been established and effectively used at various sampling sites [18], to include but not limited to the following:
(1) Washing of the tools with tap water and use of steel brush to remove adhered soil.
(2) Rinse with waste or low purity organic solvent
(3) Air- dry or acetone-dry and rinse two times with distilled water.
(4) Rinse with spectrographic grade acetone [waste may be used in step 3],
(5) Rinse twice with spectrographic grade hexane and air-dry the equipment.
(6) Packaging in clean aluminium foil or plastic bags [22, 18].
Sediment collection samplers are subjected to organic hydrophobic and hydrophilic-pollutant contamination. The cleansing protocol must involve a more exhaustive cleansing such phosphate-free detergent (Decon 90) followed by several rinses: using tap water, then with high purity water, followed with methanol and lastly with hexane. The tool must be air-dried and packed in clean aluminium foil [67]. All the waste solvents must be carefully collected for proper disposal. Apart from the use of the solvents, steam cleaning might prove effective, though steam alone may fail to provide the decontamination assurance. Some of the soil samplers are shown in figure 2.10, A-E. Sampling for this work used soil auger B (stainless steel barrel auger).

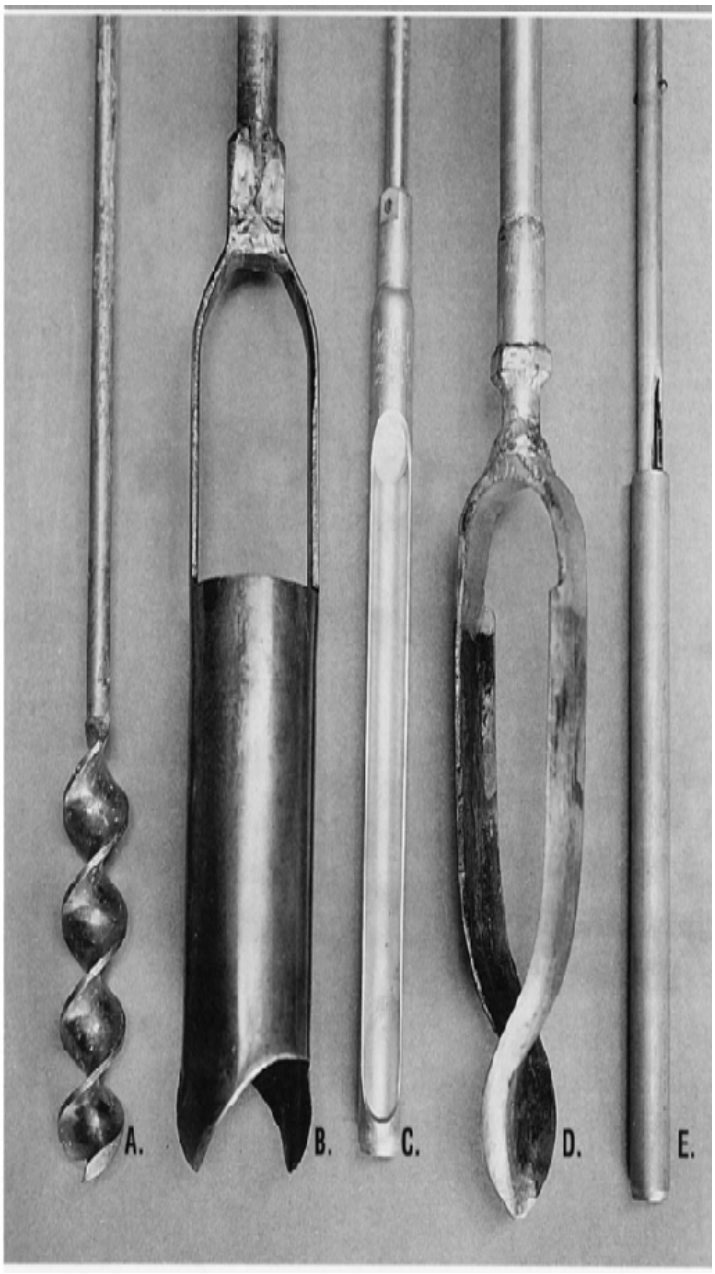


Figure 2.10 **Type of soil augers. Soil augers and tubes: A, screw or warm auger; B, stainless steel barrel auger; C, sampling tube; D, mud auger; D, peal auger [22].**

2.10.3 Sample Preservation and Containers

Preservation of samples for volatile organic compounds either begins in the time of sample analysis or in the laboratory choosing the appropriate sample containers with prescribed holding time. Holding time refers to how long the sample should be stored, after collection, preservation and preparation to obtain significant results after analysis. With EPA methods, maximum holding

time of petroleum hydrocarbons are usually 14 days, though these vary with analyte and the preservation method applied [14, 63].

Ideally, volatile organic compounds should be analyzed before 7 days after sample collection to maintain their integrity and concentrations, as they begin to change chemically, physically and biologically almost instantaneously as they are retained. Preservation is done according to regulation authority requirements.

GROs are rapidly volatilized from moist soil and sediment samples during collection, storage, transportation and analysis. The volatilization, biodegradation, oxidation and reduction lead to low-biased results. Sample preservation helps to extend the holding time for a specific time and is usually done either by the use of chemical, refrigeration or both [68, 69, 70].

Methanol (MeOH) is significantly used for preservation of soil samples containing VOCs before analysis and this must occur in the field. For high VOC concentrations (> 200 µg/kg), methanol preservation (equal amount of sample + MeOH; 1:1) in the field should be sufficient without the necessity to use other preservation techniques to obtain additional samples [69].

EPA Method 5035 recommended the use of sodium bisulphate ($NaHSO_4$) for low VOCs concentrations (< 200 µg/kg) because methanol tends to make the sample more dilute. Therefore, $NaHSO_4$ preservation is one way of effectively eliminating biodegradation of VOCs in soil samples for low level. A modification [61] for the preservation of soil, sediment and water samples by addition of trisodium phosphate dodecahydrate (TSP) instead of sodium bisulphate is gaining advantage. TSP preserves the volatile organic compounds in the sample without the threat of ether hydrolysis because the sample pH is raised above 11. Acid-preserved samples for volatile organic compounds are discouraged as dilute acid may cause hydrolysis of ethers in certain oxygenated compounds such as methyl tertiary butyl ether (MTBE).

The physical preservation technique include refrigeration where soil and sample containers are tightly sealed and iced or cooled to 4°C ± 2°C. This will reduce loss of volatile hydrocarbons and inhibit microbial action in the soil which may lead to hydrocarbon breakdown. The use of dry ice for preservation after

sampling or during shipment is not recommended [69]. The advantage of refrigeration over chemical preservation is mentioned by [66], to include:
(i) removal of addition of chemical that can affect the sample composition and integrity (2) may not interfere with analytical methods if chemicals are not added.(3) the temperature of about 4°C inactivates microbiological activity and reduces volatility of gases dissolved in the sample (4) risk hazard involved in the use of chemical is removed or reduced.
An Encore™ type sampler is another acceptable sampling technique with proven effectiveness that can be used to collect samples without field-preservation, provided the sample is extruded into methanol or sodium bisulphate within 48 hours of sample collection.
The containers commonly employed in VOCs storage are borosilicate amber glass jars, Teflon bottles with a lined lid/cap and no headspace. The maximum holding time from date of collection to analysis of semi volatiles are 40 days [71].

2.10.4 Summary of Actual Sampling Process

Sampling is an essential step in the analytical processes and related sampling terminology is recommended [65, 72] for use in order to avoid ambiguity and contradiction. Selection of a potential sampling site is followed by the drawing of a sampling plan that maps out the sampling points based on statistical approach according to the sampling strategy/pattern. Individual portion of sample collected by a single operation, herein called increment was obtained from each sampling point to produce a primary sample. A composite or aggregate or average sample is obtained when they are mixed together. By definition, a composite sample means, "two or more increments or sub-samples mixed together in appropriate proportions, either discretely or continuously, from which the average value of a desired characteristic may be obtained" [65].

A laboratory sample is then obtained from the composite or primary sample. The laboratory sample is then packed and transferred to the laboratory for pre-

treatment and characterization. Sampling procedures ordinarily end after pre-treatment exercises.

Exploratory study was carried out despite the adamant restriction to the site posed by the local militants. Collection of samples was done randomly using a stainless auger into approved containers and taken to the lab within 3 days according to the transport and handling procedures [73]. Preservation was done at 4°C in the refrigerator. Soxhlet extraction [74] commenced immediately and finished in 12 days.

The soil sampling devices were washed and always kept decontaminated between samples. Control samples were taken first at designated places far from the spill site and around the spill site. Selected samples were spiked with Pentadecane (C15) in the field to enable recoveries to be calculated after extraction and analysis (refers to chapter 5, section 4.1).

2.11 Bioremediation

Bioremediation (bio stands for organism, remediate means to correct, remove an evil or solve a problem and "bio-remediate" is to involve biological organisms to solve environmental problem such as soil and ground water. Briefly, bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Bioremediation is an effective but slow process. The first step is to identify the problem materials that must be degraded and to know which organisms will do that completely. The soil conditions are modified to favour growth of the organism or organisms. Soil aeration, the amount of water (moisture), suitable pH range, soil type and temperature may all need adjustment. For example, biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral). The acceptable range is pH 6 – 8 and thermophilic bacteria (those which survive and thrive at relatively high temperatures) which are normally found in hot springs and compost heaps exist indigenously in cool soil environments and can be activated to degrade hydrocarbons with an increase in temperature to 60°C [75, 76]. Bioremediation provides a good cleanup strategy for some types of pollution such as hydrocarbons in oil spills, halogenated organic compounds, nitrogen compounds, metals (lead, chromium,

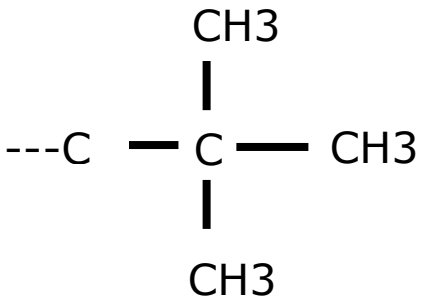
mercury etc) but not all organisms decompose every kind of substances. The first requirement is to find one or more effective micro organisms for each problem substance needing degrading.
Generally, bioremediation approaches can be grouped as in situ or ex situ. In situ bioremediation involves treating the contaminated material at the site while ex situ deals with the removal of the contaminated material which is excavated and treated elsewhere.

Bioremediation technology exploits various naturally occurring mitigation processes such as:

- Natural attenuation, in which bioremediation takes place without human intervention apart from monitoring.
- Biostimulation involves adding nutrients and other substances to soil to catalyze natural attenuation process. This uses indigenous microbial populations to remediate contaminated soils.
- Bioaugmentation refers to the introduction of a group of exogenic microbial organisms (derived from outside the soil environment) to detoxify a particular contaminant, sometimes a genetically engineered variant or altered strains [77]. This natural attenuation relies on natural conditions and behaviour of soil micro organisms that are indigenous to soil. Other examples of bioremediation technologies include bioventing, land farming, bioreactor, composting.

Bioremediation is favoured by conditions that aid microbial growth. The first step is to identify the problem materials that must be degraded and to know which organisms will do that completely. The soil conditions are modified to favour growth of the organism or organisms. Soil aeration, the amount of water, suitable pH and soil temperature may all need adjustment. Inoculation (seeding) with the correct micro-organisms may be necessary. Often the soil may need leaching after bioremediation, so general soil permeability is important.
Hydrocarbon degradation by microbial oxidation is an important environmental process because it is the primary means by which petroleum wastes are

eliminated from water and soil. Hydrocarbons vary significantly in their biodegradability and microorganisms show a strong preference for straight-chain hydrocarbons. The reason for this preference is that branching inhibits ß-oxidation at the site of the branch. The presence of a quaternary carbon (equation 14) particularly inhibits alkane degradation.



..... **2.14**

The aromatic hydrocarbon rings are still susceptible to microbial oxidation in spite of their chemical stability. Among the microorganisms that attack aromatic rings is the fungus *Cunninghamella elegans*. It metabolises a wide range of hydrocarbons ranging from C3 - C32 alkanes, alkenes, and aromatics, including naphthalene, biphenyl, and Phenanthrene [78]. Micro-organisms have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance.

The bioremediation technology most suitable for a specific site is determined by several factors, such as site conditions, indigenous microorganism population, and the type, quantity, and toxicity of contaminant chemicals present. Some treatment technologies involve the addition of nutrients to stimulate or

accelerate the activity of indigenous microbes. Optimizing environmental conditions enhance the growth of microorganisms and increase microbial population resulting in improved degradation of hazardous substances. However, if the biological activity needed to degrade a particular contaminant is not present at the site, suitable microbes from other locations, called exogenous microorganisms, can be introduced and nurtured. Other technologies being demonstrated are phytoremediation, or the use of plants to clean up contaminated soils and ground water, and fungal remediation, which employs white-rot fungus to degrade contaminants.

Depending on the site and its contaminants, bioremediation may be safer and less expensive than alternative solutions such as incineration or land filling of the contaminated materials. It also has the advantage of treating the contamination in place so that large quantities of soil, sediment or water do not have to be dug up or pumped out of the ground for treatment.

2.12 Quality Assurance and Control (QA/QC)

Quality Control is the routine use of procedures designed to achieve and maintain a specified level of quality for a measurement system. Quality assurance is defined as a set of coordinated actions such as plans, specifications, and policies used to assure that a measurement programme can be quantifiable and produce data of known quality. However, quality control differs from quality assurance in that quality control is a "system of activities to provide a quality product" while quality assurance is a system of activities to provide assurance that the quality control system is performing adequately [79].

The evaluation of soil analytes data consist in the internal, technical and laboratory good practices to control quality measurement. This process requires laboratory reagent blanks, reference standards (quality control standard), replicate (duplicate) sample analysis, standard addition (same analyte), internal standards, and surrogate (similar) analytes). Internal standards are added to each final extract solution, once all extraction, clean up and concentration steps are completed. Surrogate analytes are added to the analysis portion to provide a means of checking for every analysis, which no gross errors have occurred at

any stage of the procedure leading to significant analyte losses. Internal standards and surrogates are significantly used in analysis of organic compounds/chemicals, where analyte losses that can occur during the extraction or chromatographic steps, and the small final volumes that are frequently involved can give rise to considerable errors.

Quantification of analyte solutions can be achieved by adding internal standards, external standards, standard addition by spiking, and addition of surrogate analytes. Surrogates are not used to calculate analyte concentration directly but used to establish the recovery efficiency of a sample pre-treatment. The instrument is normally calibrated by applying the ratio of peak area (PA) or height (Pht) for analyte compared with that of internal standard (Is). The ratio of the internal standard response to that of analyte response gives the analyte concentration and is termed the relative response factor.

2.13 Risk Assessment and Safety Requirement

In recent years, many organizations have been actively addressing the question of health and safety in the workplace, partly in response to legal requirements but mostly in response to the fear of litigation and the pressures of increasing insurance premiums and other insured costs.

Undoubtedly, risk and safety procedures can never be ignored when a sample plan is developed. A proper safety protocol has to be planned and executed to protect the analyst from accidents and inevitable exposure to potentially hazardous chemicals. This document will also be useful in case of litigation and accident of workers and the need for compensation. The conception and development of "Responsible care" was the industry's response. Responsible care is the chemical industry branded program of principles and codes of practice designed to demonstrate that its members take health, safety and environmental issues into account in their everyday operations. With this in mind, the research was carried out, the risks spelt out and the relevant risk assessment documents met.

However, various risks and hazards were inherent in the process of sampling on field which include but not limited to:

- Exposure of personnel to chemicals and or reagents.
- Presence of sharp instruments for sampling
- Use of bottles and glassware
- Exposure to other danger on site
- Safety requirements should include the following:
- Wearing of protective clothing, Safety boots, safety glasses, helmets/hard hats.
- Hand gloves and ear/nose masks was worn and First aid kits put in place.

2.14 Conclusion

Many works have been done on different extraction and sampling techniques. The application of the techniques has been reviewed and the merits and demerits of each application outlined. The principles and application of the Soxhlet extraction technique was used in this work with modification and adoption of Büchi extraction apparatus. The sampling processes were carried out with typical reference to its application to spilled soil in a tropical region with high ambient and soil temperatures. Sampling plans and protocols were adequately drawn up before the sampling exercise.

The quality of every step measurement was achieved because quality control and assurance procedures were followed with health and safety measures.

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CHAPTER 3

PRELIMINARY METHOD DEVELOPMENT

3.1 Overview

In this chapter, a brief overview of the analytical instrumentation and profile of the BTEX and some liquid petroleum hydrocarbon is described. The results shown cover the instrument calibration, calibration curves and retention times to characterize the analytes in the method developed. The aim was to establish a concentration range for analysis of the GRO (BTEX) and the DRO in the extracts of petroleum-contaminated soils applying optimized methods.

3.2 Gas Chromatography

Gas chromatography (GC) is one of the most powerful, popular, unique and readily versatile analytical techniques used for the separation, identification, and quantitative assay of compounds in the vapour state. The popularity of GC is absolutely centred on high selectivity, sensitivity, high resolution combined with good accuracy and precision in a wide dynamic concentration range [1, 2, 3]. Gas chromatography is deficient in that it cannot render a definitive proof of compound as separation progresses while detection and identification is limited to the retention time for most GC detectors [2].

The following constitute the components of a gas chromatograph:

- The carrier gas (mobile phase)
- Sample injection chamber
- A column (impregnated with the stationery phase) in an oven
- Detector
- Data recording and display system.

It involves an injected sample being vaporized and transported through the column by flow of inert, gaseous mobile phase (carrier gas). Separation of organic component (analyte) is achieved on the basis of partioning of the analyte between the gaseous mobile phase and the stationary phase which is a non-volatile liquid immobilized on a capillary tubing or an inert solid packing [4, 5, 3, 6].

The basic component of a typical gas chromatograph is illustrated schematically in figure 3.1 and the photograph of Varian CP- 3800 GC/FID in figure 3.2 below.

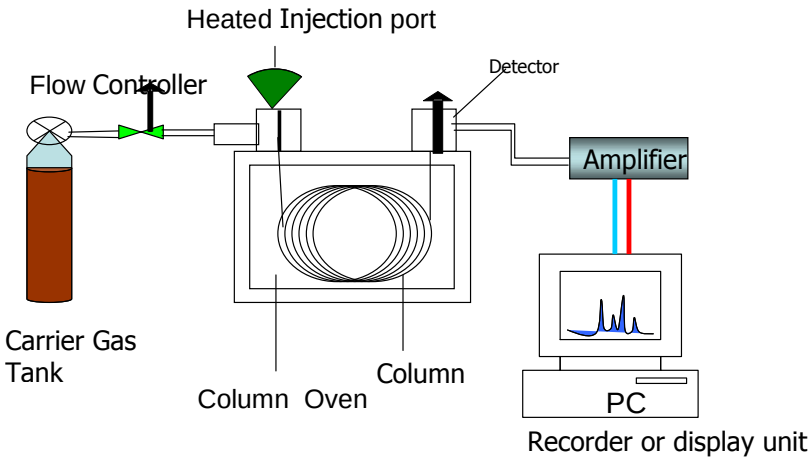


Figure 3. 1 Schematic diagram of a gas chromatograph

The most common injection method is the use of micro syringe to administer sample through a rubber septum into a flash vapourizer port at the head of the column contained in a thermostat–controlled oven. The temperature of the sample port should be about 50°C higher than the boiling points of the least volatile component of the analyte.

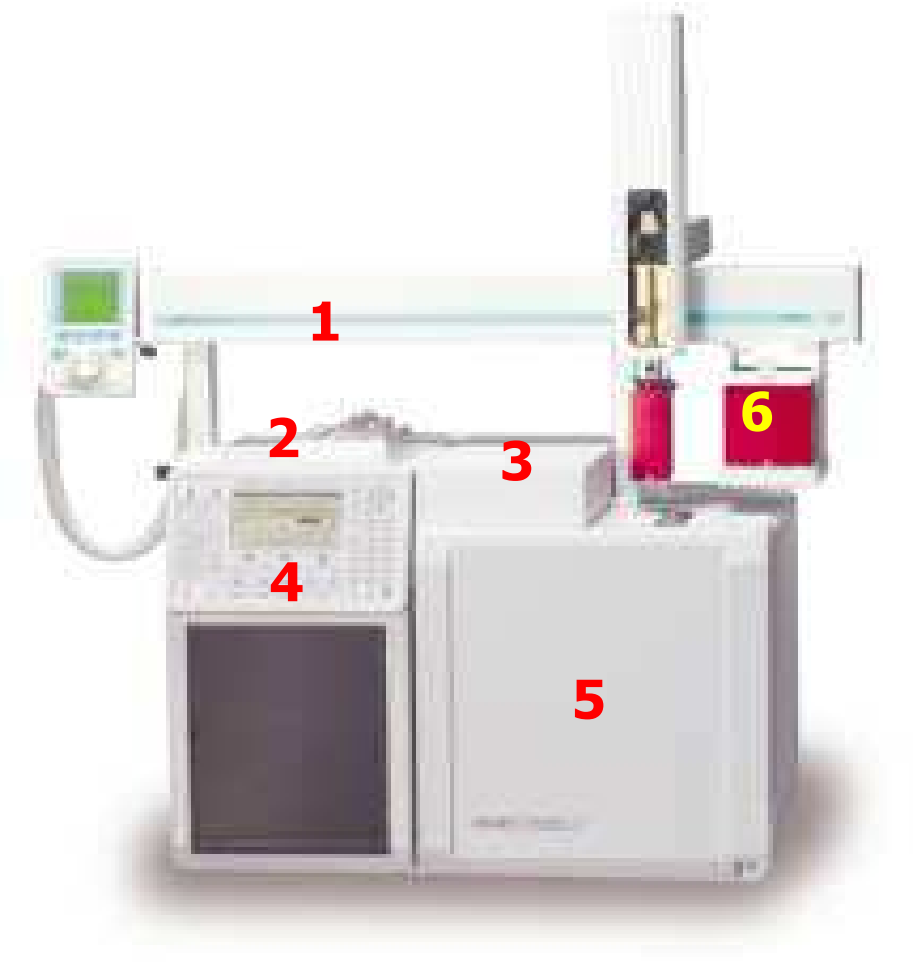


Figure 3.2 Photograph of Varian CP-3800 GC-FID Instrument. 1: CombiPAL automated sampler, 2: Sample tray one, 3: Sample tray two, 4: GC control panel, 5: Oven-column unit, 6: Agitator box.

Separation or elution is dependent on the boiling points of the samples with low boiling components eluting first before the higher boiling ones. The analytes elute through the column into the detector, which gives an electrical signal and enters the data recording and display unit. This signal generates the chromatogram.

3.2.1 Theory of Gas Chromatography

In gas chromatography, a very small amount of the sample to be analyzed is drawn up into a syringe needle and injected into a hot injector port of the gas chromatograph. Components of the mixture are vaporised into the gas phase and a carrier gas, such as helium or hydrogen, flows through the injector and moved the gaseous components of the sample through the GC column unto a detector. The injector is set to a temperature higher than the analytes boiling points and placed in an oven whose temperature can be controlled. It is within the column that separation of the components takes place. Molecules partitioned between the carrier gas (the mobile phase) and the high boiling liquid (the stationary phase) within the GC column [5].

Chromatographic separation process is based on the difference in the surface interactions of the analyte and eluent molecules. Therefore the differential distribution into the stationary phase allows the components to be separated in space and time. This time is called the retention time (t_R).

3.2.2 Retention Parameters

Retention time (t_R): This is the time required for the analyte peak to appear [4] which is equal to the total time taken for a particular compound to travel through the column after injection until it reaches the detector. High temperatures and high flow rates decrease the retention time. Retention time is inversely proportional to the eluent flow rate, $\mathbf{F_C}$. Retention time is generally reported in minutes.

Dead time (t_m) is the amount of time the unretained compound travels in the column.

Adjusted retention time, t'_R – additional time for solute to travel the length of the column, beyond the time required by unretained solvent. It is the time

difference between the dead time and the retention time for a compound (refer to figure 3.3).

$$t'_R = t_R - t_m$$

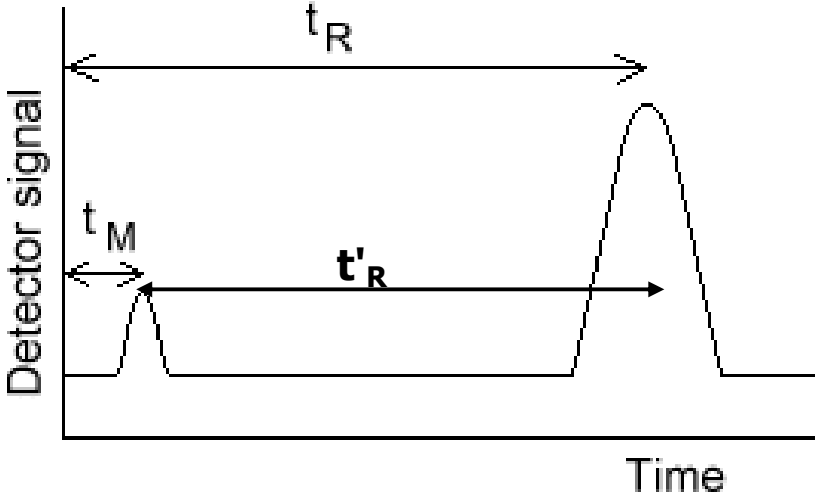


Figure 3.3 A chromatographic illustration of the retention parameters

Retention volume, (V_R) – volume of a mobile phase needed to elute a particular component from the column.

$$V_R = t_R \times F_C$$

$$t_R = V_R / F_C$$

Partition ratio or Capacity factor (K') - also called a retention factor is the dimensionless measure of the column's retention of a compound. The K' for each peak in a particular chromatogram is defined as:

Capacity factor: $$K' = \frac{t_R - t_m}{t_m}$$

The longer a component is retained in the column, the greater the capacity factor, k'. If the solute is not adsorbed and spend all the time in mobile phase

$$\mathbf{t_R = t_m}$$

$$\mathbf{K' = 0}$$

If the solute spends equal time in mobile and stationary phases

$$\mathbf{t_R = 2t_m}$$

$$\mathbf{K' = 1}$$

This means that:
K' = (time solute spends in stationary phase) / (time solute spends in mobile phase).

Resolution – provides another measure of how well species have been separated in minimum analysis time. The resolution of two species, A and B, is defined as:

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B} \quad \text{..... 3.1}$$

Where:

$(t_R)B$ and (t_R) B are separation between peaks (retention times)

W_A and W_B are the widths at the bases of the peaks

Optimum column efficiency is achieved when the plate height, H , is minimum at a given column length. Resolution is dependent on characteristics of the compound, selectivity capacities of column and column efficiency (band broadening effect) [7] as shown in section 3.2.3 below.

3.2.3 Separation Efficiency of Column

Chromatographic separation of a compound depends on two basic factors namely (i) peak broadness and (ii) difference in elution times between peaks. The longer the solute is retained in column, the broader the peak is. The wider the peaks, the poorer the separation. The higher the column band broadening, the smaller the number of components that can be separated in a given time.

The sharpness of the peak is an indication of how good or efficient a column is. Separation efficiency in chromatography is expressed in terms of the number of theoretical plates, N in the column [4]. Theoretical plates, sometimes known as height equivalent to a theoretical plate (HETP) are dimensionless numbers representing single equilibration steps during separation. The number of theoretical plates N, in the columns is expressed as:

$$N = \frac{L}{H}$$

Where: L = column length

H = plate height

And plate height is given as:

$$H = \frac{L}{N}$$

The most useful expression for number of plates on column, N can be obtained from a chromatogram from the expression written below:

$$N = 16\left(\frac{tR}{w}\right)^2 = \left(\frac{tR}{\sigma}\right)^2 \quad \text{..... 3.2}$$

Where: t_R = the retention time of the peak

w = width at the base of peak

σ = w/4

If the peaks are reasonably symmetric, it can be assumed that they are Gaussian in shape and by using the more practical peak width at half height, $W_{1/2}$ rather than with at the base, N becomes:

$$N = 5.54\left(\frac{t_R}{w_{1/2}}\right)^2 \quad \text{.....} \quad \textbf{3.3}$$

Where: $W_{1/2}$ is width at half peak

N varies depending on the compound as well as the stationary phase material. It also depends on the flow rate and the column length. A column does not have a single N value and it is good practice to specify the column conditions and the compound used to determine N. The efficiency increases as the number of theoretical plates increases, thereby increasing the column's ability to separate two closely eluting peaks.

3.2.4 Plate Theory

The column is considered to be separated on to a number of plates on which the equilibrium of the solute with the mobile and stationary phases takes place. The whole length of the column is divided by this number of the theoretical plates to give the height of the theoretical plate (H). The efficiency of the column depends on the number of theoretical plates available. The smaller the plate height the narrower the band. The van Deemter equation shows how the column and linear velocity affect the plate height. The equation relates plate height, H, to the average linear velocity, μ and the band broadening parameters [7] as expressed below:

$$H = A + \frac{B}{\mu} + C\mu \quad \text{.....} \quad \text{Van Deemter Equation for plate height} \quad \textbf{... 3.4}$$

Where μ = linear velocity (linear flow rate), A, B, and C are constants for a given column representing:

A = Eddy diffusion (multiple paths)

B = Longitudinal or molecular diffusion

C = Interphase mass transfer (resistance to mass transfer)

Van Demeter equation indicates that band broadening mechanisms are independent of flow rate, average linear velocity is preferred to flow rate since it is directly related to speed of analysis [7].

The A term is independent of velocity and represents eddy mixing. A is smallest when the packed column particles are small and uniform. The B term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by μ. The C term is due to kinetic resistance to equilibrium in the separation process. [5, 7 8]. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase.

The Van Demeter equation can further be expanded to:

$$H = 2\lambda d_p + \frac{2\gamma D_g}{\mu} + \frac{8}{\pi^2}\frac{k d_f^{\,2}}{(1+k)^2 D_l}\mu \quad \text{..... } \mathbf{3.5}$$

H = Plate height

A = 2 λ d_p = Eddy diffusion (multiple flow path)

Where: λ = factor characteristic of packing (particle shape)

d_p = average particle diameter

B/μ = Longitudinal or molecular diffusion (diffusional broadening of band)

Where: γ = factor for irregularity of inter particle spaces

D_g = diffusion coefficient of compound in gas (mobile phase)

C/μ = Effect of mass transfer between phases (Interphase mass transfer term)

Where: D_l = diffusion coefficient of compound in liquid (stationary phase)

k = retention factor (capacity ratio)

d_f = liquid phase effective film thickness

μ = linear gas velocity

An illustration of the application of van Deemter equation is shown in figure 3.4

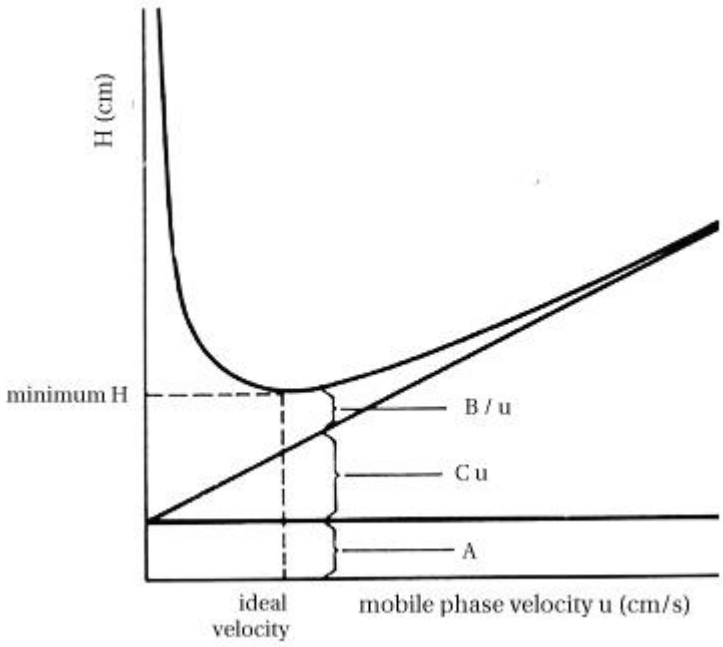


Figure 3.4 A plot of plate height vs. average linear velocity of mobile phase (Reproduced from [4].

A. Eddy diffusion

Efficiency could be increased if term A be reduced by reducing the particle diameter (which will lead to the increasing of the column back pressure) and by

narrowing the size distribution. The mobile phase moves through the column which is packed with stationary phase. Solute molecules will take different paths through the stationary phase at random. This will cause broadening of the solute band, because different paths are of different lengths.

B. Longitudinal diffusion

The longitudinal diffusion (along the column long axis) leads to the band broadening of the chromatographic zone. The concentration of analyte is less at the edges of the band than at the centre. Analyte diffuses out from the centre to the edges. This causes band broadening. If the velocity of the mobile phase is high then the analyte spends less time on the column, which decreases the effects of longitudinal diffusion.

C. Resistance to mass transfer

A certain amount of time is required for the analyte to equilibrate between the stationary and mobile phase. If the velocity of the mobile phase is high, and the analyte has a strong affinity for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the stationary phase. The band of analyte is broadened. The higher the velocity of mobile phase, the worse the broadening becomes.

3.2.5 Carrier gas

The carrier gas must be available in pure form and chemically inert. The commonly used carrier gases include helium (He), nitrogen (N_2), argon (Ar) and carbon dioxide (CO_2). Helium, though expensive is commonly used, while Nitrogen is cheapest but less effective. The choice of the carrier gas is often dependent on the type of detector [4, 5]. The carrier gas system also contains a molecular sieve to remove water and other impurities such as CO_2, H_2, N_2, O_2 and CH_4. The carrier gas flow was quantified by either linear velocity (in cm/sec) or volumetric flow rate expressed 1 mL/min [8]. The flow rate depends on column diameter while linear velocity is independent of the column diameter.

3.2.6 Columns

The GC column is the heart of the system. There are two general types of columns. The packed columns and open tubular or capillary columns. Packed columns were the first type and are probably not frequently used nowadays.

Capillary columns, commonly used in today's work are more efficient than packed columns. Capillary columns require smaller amounts of injected analytes compared to packed columns and capillary columns are much easily overloaded. For example, an analyte A can be easily separated with mass of 153 µg A using packed columns while capillary columns will do separation of A with 15.3 µg A. Actually, the diameter and chromatographic film of modern capillary gas chromatographic columns dictates the size of injected samples. This sample size requirement initially meant that if samples contained components that were too concentrated for a capillary chromatographic analysis, the sample had to be diluted before it could be analyzed; otherwise the column will be overloaded by those high concentrated components leading to change in retention time, peak distortion and broadening. Ideally, there are three major types of open tubular columns: WCOT (Wall-Coated Open-Tubular), SCOT (Support Coated Open-tubular), PLOT (Porous Layer Open-Tubular); depending on the type and material used in coating the stationary phase. The resolution efficiency is generally in the order: WCOT>SCOT>PLOT. SCOT has less efficiency but higher sample capacity than WCOT [4, 7, 9] as summarized in table 3.1.

Table 3.1 Comparison of Column properties.

Column Types	Length (m)	Internal diameter (mm)	Efficiency (N/m)	Capacity (ng/peak)
PACKED	1-6	2-4	500-1000	10
WCOT	10-100	0.20-0.75	1000-4000	10-1000
PLOT	10-100	0.5	600-1200	10-1000

Ewing's Anal. Instrumentation Handbook [9]

The choice of stationary phase is critical for achieving selectivity. The stationary phases are high molecular weight, thermally stable liquid polymers such as polysiloxane and polyethylene glycols (carbowax). A typical capillary column has a length of 15-60 meters and 0.25-0.32 mm ID. A typical packed column is 15-30 meters long and 2.2 mm ID [5, 9].

Fused silica open tubular (FSOT) is another flexible column with much thinner walls than the capillary columns with enforced strength by the polyamide coating. Comparison of the columns helped to select the type of capillary column used in this work.

3.2.7 Sample Injector Mode

A gas chromatographic sample injector is a hollow, heated, glass-lined cylinder where the sample is introduced into the GC. Therefore, for optimum column efficiency, the sample should not be too large, and should be injected into the GC column by micro syringe through a silicon rubber septum into a heated port containing silanized glass liner. The vaporized sample goes into the GC column. In order to achieve efficiency for capillary GC, split/splitless injection was used as shown in figure 3.5. The most popular injection inlet for GC is the split / splitless injector.

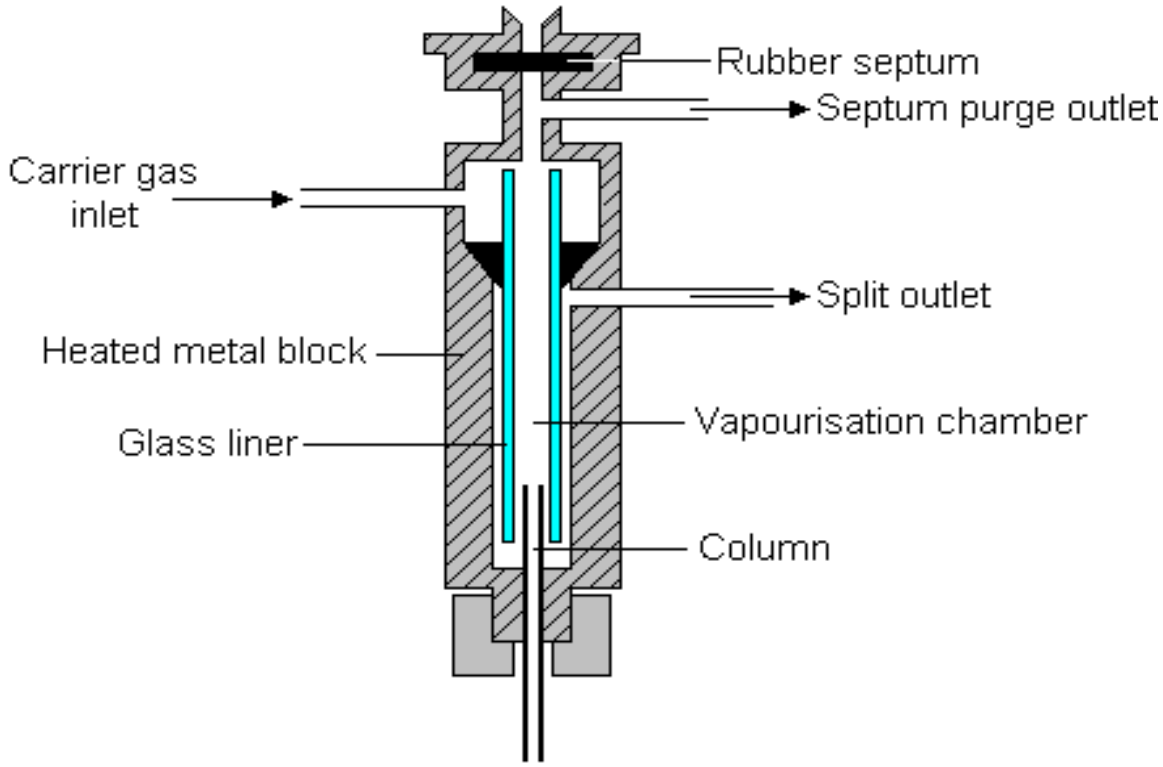


Figure 3.5 Split/splitless Injector [10])

3.2.8 Split / Splitless mode

The injector can be operated in one or two modes: split or splitless. Injector is operated in the split mode if the analyte of interest constitute >0.1% of the sample. If the concentration of analyte is high, say 30-µg analyte µL, a split injection is used for capillary column and 1 µl is injected. In this mode, the amount of the sample reaching the column is reduced to obtain narrow band widths and avoid column overloading [5]. In modern GC instrument, the amount of injected analyte into the column is controlled instantly by a flick of software that may be stored in the analytical method (known as split ratio).

When in the split mode, some of the sample injected by the sample syringe will get vaporized and escape through the vent as gas in the injector as it does so. It is so called spilt injection because the lost sample will not go on to the

column but to waste and so split away. For instance, if the split ratio is 50 to 1, it means, for every 50 units of gaseous sample that are thrown away to waste, only 1 unit enters the column.

When the sample contains very small (trace) amount of analyte or analytes that are less than 0.01% of the sample, the splitless mode is adopted. In this mode, all the analyte mass in a 1 µL injection that goes onto the column is not split. This proved to be the most sensitive method that was adopted during the instrumentation method development in this report.

3.2.9 Detectors

Separated components of a mixture of compounds from the chromatographic column must be detected at the outlet so that they can be adequately identified and measured. Usually, detectors do not identify but indicates that something has emerged from the column. However, Mass Spectrometer (MS) and Fourier transform infrared spectrometer (FTIR) are the two detectors that can identify compound for qualitative analysis [2, 5]. The characteristics desirable of most chromatographic detectors include: good sensitivity and selectivity, linear response to solute over several magnitude, good stability and reproducibility, adequate temperature range from room temperature to above 350°C, high reliability and short time of response, non-destructive of sample, response to solutes in similar manner. No one detector that is widely used today possesses all these characteristics [3, 4, 5]. Detectors could be classed depending on their characteristic functions.

A non-selective detector responds to all compounds except the carrier gas. A selective detector responds to a range of compound having similar or common chemical or physical properties. A specific detector responds to a single chemical compound.

Detectors can also be categorized as:

- Concentration dependent
- Mass flow dependent

In a concentration dependent detector, the signal is proportional to the solute in the detector and does not destroy the sample dilution. Mass flow dependent detectors give signal that relates to the rate at which solute molecules enter the

detector and usually destroys the sample [3, 5]. The most widely used detectors include but not limited to:

(i) Flame Ionization Detector (FID)

(ii) Mass Spectrometer (MS)

(iii) Thermal Conductivity Detector (TCD)

(iv) Electron Capture Detector ECD).

(v) Photoionization Detector (PID)

A typical Flame Ionization Detector (FID) was fitted in the GC for this work (figure 3.6) The Flame Ionization detector (FID) is the most widely used and general detector sensitive for gas chromatographic analysis of organic compounds except formaldehyde, formic acid and fully halogenated compounds. The effluent from the column is mixed with hydrogen and air, and ignited. Most organic compounds burning in the flame produce ions (cations such as CHO^+) and electrons which can conduct electricity through the flame and form the basis of a very sensitive detector called FID.

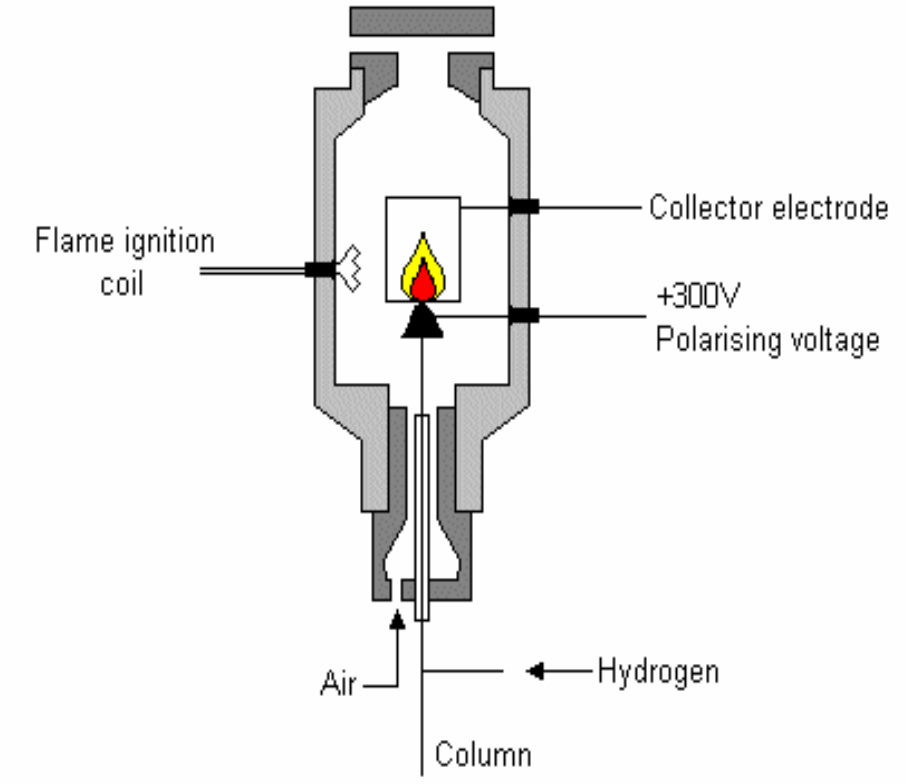


Figure 3.6 A typical Flame Ionization Detector (FID) [10]

A huge electrical potential is applied at the burner tip with the collector electrode positioned above the flame, and the current resulting from the pyrolysis of the organic compounds is then measured. FID is robust, easy to use and has high sensitivity, large linear response range and low noise or stable baseline. FID is mass sensitive rather than concentration sensitive and derives the advantage that changes in the mobile phase flow rate or temperature fluctuations do not affect the response of detector. Its disadvantage is that it destroys the sample and lacks response to carrier gas impurities, CO_2, H_2O and inorganic solutes [7, 9] thereby providing no signal without analyte. FID was used for this work because of its suitability and applicability to petroleum extractable hydrocarbons.

3.2.10 Mass Spectrometer Detector

The mass spectrometer is one of the most powerful detectors for both qualitative and quantitative analysis of compounds in gas chromatography. GC/MS combines the strengths of gas chromatography and mass spectrometer to give most versatile and useful detector. Mass spectrometer measures the mass-to-charge ratio (m/z) of ions that have been produced from the sample solution.

Separation of sample into relative pure components is carried out by the GC while the MS renders qualitative information of the separated components though a combination of ionization, difficult separation of compounds with similar mass fragmentations resulting in interpretation of spectra, identification of molecular structure and molecular weight [4, 5, 3, 6]. The major components of MS include: the ionization source of analytes, analyzer separates the analyte according to m/z ratio and gives electrical signals at the detector.

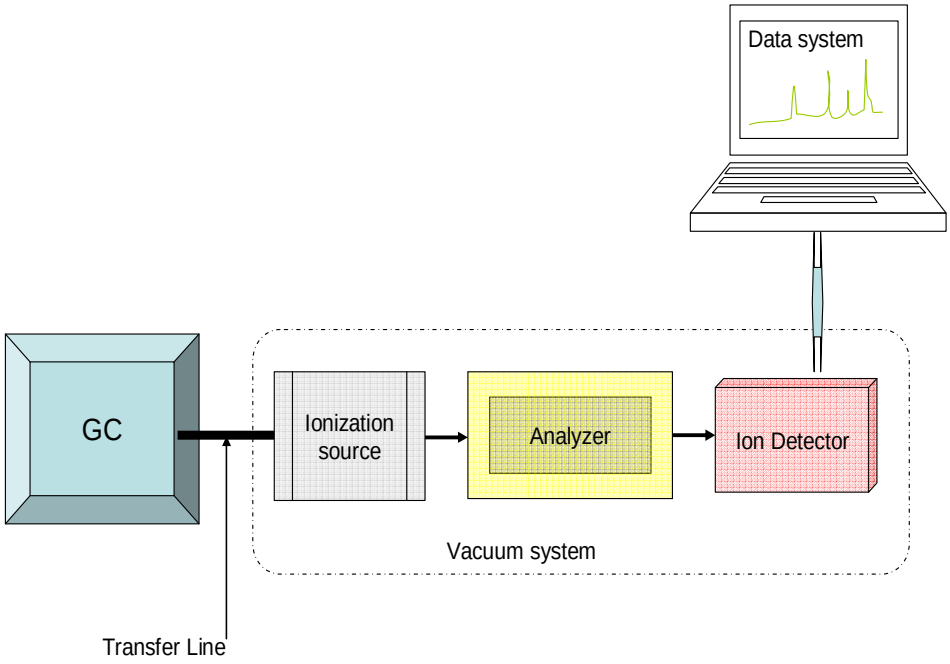


Figure 3.7 Block diagram of GC incorporated with MS

The GC column introduces the sample through the transfer line into the ionization source chamber which fragments the molecules and converts them to ions. These ions proceed to the analyzer to be separated according to their charge-to-mass (m/z) ratio. The separated ions finally impinge on the detector to generate an electrical signal that are recorded and plotted by the data system. Two ionization methods are most widely used in GC-MS analysis; Electron Impact (EI) and Chemical Ionization (CI). CI, being a modification of EI, gives less fragmentation compared to EI [11].

3.3 Application of Gas Chromatography to soil and sediment in Environmental analysis

The popularity of GC is based on a healthy, favourable and growing measurement technique with expanding influence in innovative applications

such as emerging organic pollutants (VOCs, PAHs, Polychlorinated biphenyls (PCBs), pesticides etc), oil-spill analysis, pharmaceutical and food analysis [1]. The role of GC-MS as a more useful and powerful analytical technique in compositional analysis in the petroleum industry has been extensively covered by [12]. The work applied Field ionization (FIMS) mode to produce the needed spectra without fragmentation. Guardia-Rubio, [13] found GC-MS-MS a highly selective and efficient detection method for the determination of pesticides in natural products such as virgin olive oil. Analysis of petroleum contaminated soil and sediments using GC-MS has been adopted for the analysis of hydrocarbons by [14, 15].

3.3.1 Instrumentation and Apparatus

Two types of GC-FID instrument was used in this preliminary study.
The system consisted of a Varian BV (from Varian instruments, Middelburg, The Netherlands) CP-3800 gas chromatograph coupled to the FID detector equipped with an automatic sampler CTC Analytics CombiPAL and the 1177 split/splitless front injector. The GC capillary (WCOT) column was a non polar, CP-Sil 8 CB Low Bleed/MS polymer ; 30 m long, 0.25 mm inside diameter (id), 0.39 mm outside diameter (0d), 0.25 µm film thickness, coating of 95% methyl, 5% phenylpolysiloxane [30 m x 0.39 mm x 0.25 µm].

Daily checks were routinely conducted, before the commencement of any analysis to ensure that the system was perfect to deliver high quality and reproducible results. During my training and familiarization with the GC-MS instrument, daily checks for water/ air 19/18 ratio, column and cold trap blanks, ionization check for filament etc were performed.

Unfortunately, the GC-MS was not accessible in this training and method development exercise principally due to full time allotment and use of the instrument by many analysts. Individual booking and analysis times often involved incessant change of GC columns and the GC parameters thereby reducing efficiency of work. Therefore, the availability and suitability of GC-FID for the separation of the petroleum contaminants made it paramount at this stage and throughout this research work.

3.3.2 Materials and Reagents

The following chemicals were used during method development. The analytes in consideration were characterized and used to calibrate the instrument. The properties of the compounds were outlined and calibration curves prepared for the following:

- Benzene – 99.9% HPLC grade supplied by Sigma Aldrich, D89555-Steinheim, Germany.
- Toluene – 99.8%, Analytical reagent grade bought from Fisher Scientific Chemicals, UK Limited, Bishop Meadow Road, Loughborough, UK.
- Ethyl benzene – 99.8%, HPLC grade, supplied by Sigma Aldrich, UK.
- o-xylene – 97% anhydrous, procured from Sigma Aldrich Chemical Co, Inc, box 355, Milwaukee, USA.
- Methanol – 99.9% HPLC gradient grade obtained from Fisher Scientific, UK.
- Hexane/cyclohexane, 99.9% HPLC grade from Sigma Aldrich, Gillingham, UK.
- Heptane - 99% GC grade, Cat Log # H6, 70-3.
- Undecane (C11) – GC grade
- Gas oil (diesel) – 0.5% obtained from the stock in the lab.
- Pentadecane (C15) – 99% from Sigma Aldrich, UK.

Methanol, hexane and heptane were the solvents, which were all of analytical grade supplied by Sigma-Aldrich.

Table 3.2 Properties of Benzene

Analyte	Benzene
Appearance	Clear, Colourless liquid
Solubility, 25°C (g/L)	Negligible about 1.79 in water.
Specific gravity (g/mL)	0.87
Boiling point (°C)	80.1
Molecular weight (g)	78.11
Molecular formula	C_6H_6
Molecular structure	
Principal hazards and safety	Carcinogen, very flammable and should be handled in a fume cupboard with safety glasses and protective gloves.
CAS Number	71-43-2

Table 3.3 Properties of Toluene

Analyte	Toluene (methylbenzene)
Appearance	Colourless liquid
Solubility, 25°C (g/L)	Slightly soluble in water.
Specific gravity (g/mL)	0.865
Boiling point (°C)	111.0

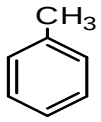
Molecular weight (g)	92.14
Molecular formula	C_7H_8
Molecular structure	
Principal hazards and safety	Toxic if swallowed and harmful on contact with skin. Very flammable.
CAS Number	108-88-3

Table 3.4 Properties of Ethylbenzene

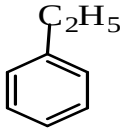
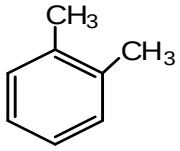
Analyte	Ethylbenzene (methylbenzene)
Appearance	Colourless liquid
Solubility, 25°C (g/L)	Very slightly soluble in cold water.
Specific gravity (g/mL)	0.867
Boiling point (°C)	136
Molecular weight (g)	106.17
Molecular formula	C_8H_{10}
Molecular structure	
Principal hazards and safety	Harmful through skin contact or if ingested or inhaled. Causes eye irritation.
Principal hazards and safety	Harmful on prolong exposure to skin and if inhaled. Adequate ventilation and safety glasses needed when in use.
CAS Number	100-41-4

Table 3.5 Properties of o-xylene

Analyte	O-xylene (1,2-dimethylbenzene)
Appearance	Clear, colourless liquid
Solubility, 25°C (g/L)	Sparingly soluble in water.
Sp. gravity (g/mL)	0.88
Boiling point (°C)	144.4
Molecular weight (g)	106.17
Molecular formula	C_8H_{10}
Molecular structure	
Principal hazards and safety	Harmful on prolong exposure to skin and if inhaled. Adequate ventilation and safety glasses needed when in use.
CAS Number	95-47-6

3.3.3 Calibration curve

Calibration or standard curve forms a very important part of analytical procedures and is achieved by the use of high grade chemical standards. The essence of calibration in analytical work is to ensure accuracy and reproducibility of the results.

A calibration curve is a graph that shows the detector response as a function of analyte concentration [5, 2]. Working standard solutions of known concentrations were prepared and injected into the GC column. The peak heights, usually of replicate measurements from the resulting chromatograms were used to construct the calibration curve. Therefore, analytical methods are

validated by demonstrating that accurate results are obtained from the above process. Calibration curves were generated over the range of 10 ppm to 100 ppm for seven standards including diesel oil.

However, the following approaches were adopted in the preparation of the standard solutions.

Firstly, stock solutions of the gas oil, undecane, pentadecane, benzene, toluene, ethylbenzene and o-xylene were prepared in methanol, DCM and or hexane solvent followed by dilution of the stock solutions to make standards of desired low concentrations (Appendix I). Table 3.6 gives a summary of the properties of the tested analytes.

Table 3.6 Summary of the properties of the tested analytes

Compound	Mol. Wt (g/mole)	Boiling Point (°C)	Specific gr. (gcm^{-3})	Molecular Formula	CAS No.
Methanol	32.04	64.6	0.79	CH_3OH	67-56-1
Undecane	156.31	196	0.74	$C_{11}H_{24}$	1120-21-4
Pentadecane	212.42	269	0.77	$C_{15}H_{32}$	629-62-9
Benzene	78.12	80.10	0.87	C_6H_6	71-43-2
Toluene	92.14	111.0	0.87	C_7H_8	108-88-3
Ethylbenzene	106.17	136.2	0.87	C_8H_{10}	100-41-4
o-xylene	106.17	144.4	0.88	C_8H_{10}	94-47-6

3.3.4 Instrument Parameters

Daily checks, test mixtures and reagents of known concentrations such as the BTEX and the hydrocarbons were run with instrument parameters below: Both isothermal and programmed modes were tested for efficiency comparison.

Table 3.7 Instrument Parameters

Isothermal

CP-3800 GC Parameters	
Carrier gas	Helium
Oven temperature	150°C Isothermal
Column flow rate	1.0 mL min^{-1}
Column stationary phase	CP-Sil 8 CB 5% phenyl,95% methyl
Column length	30m
Column inside diameter	0.25mm
Column outside diameter	0.25mm
Column film thickness	0.25 µm
Injector Temperature	300°C
Split flow ratio	100.1
Run time	30min

Detector parameters

Temperature	270°C
Hydrogen (H2) flow rate	30 mL mim^{-1}
Air flow rate	300 mL mim^{-1}

Table 3.8 Temperature programming:

Temperature (°C)	Ramp °C/min	Hold (Min)	Total (Min)
40	0.0	2.00	2.00
240	8.0	5.00	29.50
Column flow rate	1.0 mL/min		
Detector parameters			
Hydrogen flow rate		30 mL/min	
Air flow rate		300 mL/min	
Split ratio		20:1	

3.4 Development and Optimization of GC-FID method

Development of GC-FID methods for the analysis of benzene, toluene, ethylbenzene and o-xylene were carried out. The optimization of previous methods took place in order to obtain maximum sensitivity and yield of these analytes in the method applied. The GC-FID optimization process involved several adjustments in the method with different column temperatures, column flow rates, split ratios (split/splitless), head pressure as applied to both isothermal and temperature programming.

A range of column oven temperatures were tried (40°C-270°C) to establish maximum sensitivity at reduced retention time. However, because of the optimization for the liquid injection, the following were considered the appropriate GC- FID method for the analytes.

Table 3.9 GC parameters for isothermal temperature run.

Column Type :	95% methyl, 5% phenylpolysiloxane capillary column CP-Sil 8CB: 30mx0.25mmx0.25µm
Column oven:	60°C isothermal
Flow pressure:	2.0mL/min (column)
Sample injection volume:	1µl
Split ratio:	50:1
Front injector (1177) tempt:	270 °C
Detector temperature	300 °C

Table 3.10 GC parameters for temperature programme: Temperature ramp.

Temperature °C	Rate °C/min	Hold time	Total min
50	-	2.0	2.0
100	5	3.0	15

The same column and conditions applied for isothermal programme were adopted for temperature programming (ramping).

Chromatograms of both isothermal and ramp temperature programming were run and methods compared.

Table 3.11 Comparison of retention times and peak areas in programme modes.

Temperature programme was done on another column programme different from figure 3.12.

Compound	**Peak #**	**Retention time**	**Peak area**
Methanol	1	2.48	-
Benzene	2	3.85	688
Toluene	3	5.68	637
Ethylbenzene	4	8.19	687
O-xylene	5	10.18	641

Figure 3.12 Comparison of retention times and peak areas in Isothermal temperature programme

Compound	**Peak #**	**Retention time**	**Peak area**
Methanol	1	2.47	**-**
Benzene	2	3.39	481
Toluene	3	5.32	452
Ethylbenzene	4	8.76	475
O-xylene	5	10.67	452

3.5 Calibration of the standards

Injection of different concentrations of the analytes was carried out in order to prepare the calibration graphs of their standards. However, to optimize better sensitivity on the ideal method, some adjustments were made on the column oven temperature to accommodate the concentration range of all the analytes in the method. Due to this adjustment the run time was also changed. Therefore, the ideal method applied to prepare the various calibration curves is presented below. Calibration range was determine by preliminary anlysis.

Table 3.13 The optimized method for the calibration of standards

Temperature °C	Rate °C/min	Hold time	Total min
40	-	10.0	10.0
100	6	10.0	30.0
Split ratio	25:1		
Run time:	30 minutes.		

3.5.1 Calibration curve for Benzene

1000 ppm of benzene stock solution was prepared by taking 11.5µl of benzene and making it to mark in a 10 mL volumetric flask with methanol solvent using ependorf micropipettes.

By careful and appropriate dilution of the stock solution, 10, 20, 30, 40, 50, 60, 70, and 100 ppm standard solutions of benzene were prepared (Appendix 1).

Table 3.14 Calibration data for benzene standard showing concentration and chromatographic peak heights

Conc. (ppm)	**Peak Area** (mV s) 3 injections			**Average Peak Area** (mV s)	**Retention Time** (s)
	1	2	3		
10	3.89	3.73	3.93	3.85	3.76
20	7.24	7.75	7.71	7.57	3.76
40	15.6	15.3	15.7	15.53	3.76
70	27.5	27.0	27.2	27.23	3.76
100	39.7	40.5	37.7	39.30	3.76

NB: Calibration was determined by preliminary analysis of the tested analytes.

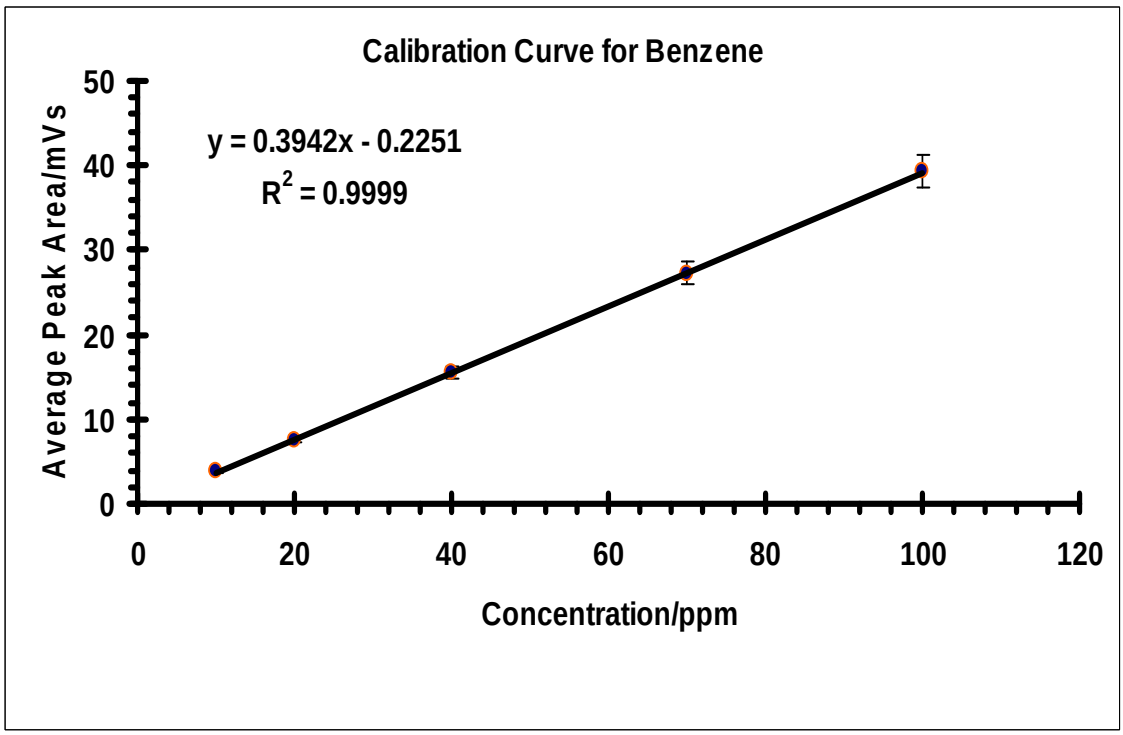


Figure 3.8 **Calibration curve for benzene over a concentration range of 10 to100 ppm obtained by linear least-square regression analysis. Correlation coefficient (R^2) is 0.99. Error bars denote 95 % confidence level and is based on triplicate measurements**

3.5.2 Calibration curve for Toluene

Standard concentrations of toluene were similarly prepared as for benzene for concentration range of 10-70 ppm (Appendix 1).

Table 3.15 **Calibration data for toluene standard showing concentration and chromatographic peak heights**

Conc. (ppm)	**Peak Area** (mV s) 3 injections			**Average Peak Area** (mV s)	**Retention Time** (s)
	1	2	3		
20	10.9	10.6	10.3	10.6	6.91
30	18.4	17.8	18.1	18.1	6..91
40	22.6	22.3	22.1	22.3	6.91
50	32.1	31.7	30.1	31.3	6.91
60	35.8	34.4	34.8	35.0	6.90
70	43.5	43.9	42.1	43.2	6.91

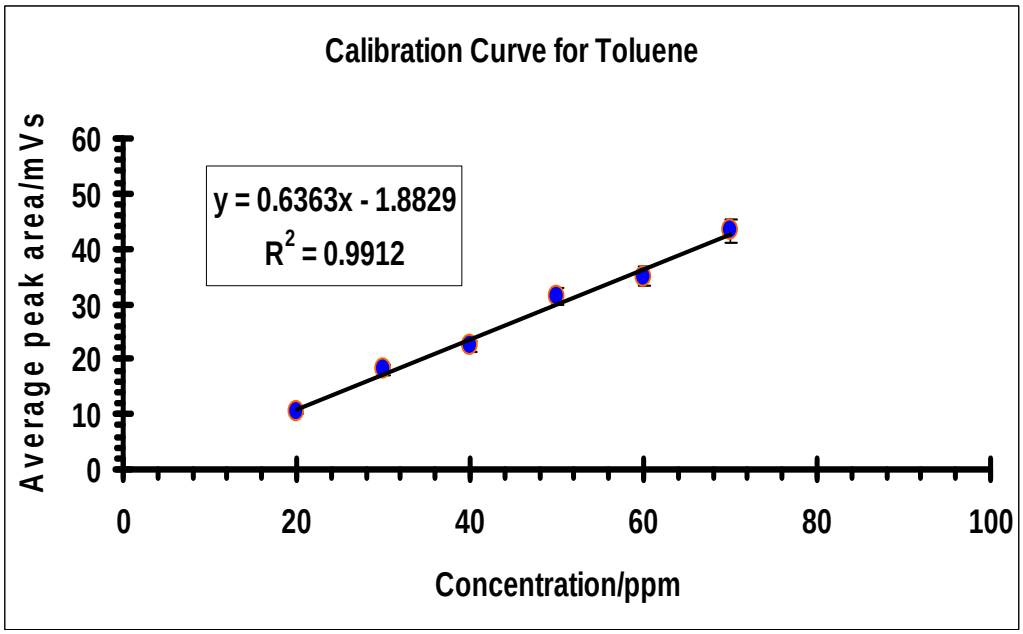


Figure 3.9 **Toluene calibration curve for concentration range 10-70 ppm. The linear least-square regression analysis gave 0.99 correlation coefficient (R^2) at 95 % confidence level of triplicate measurements**

3.5.3 Calibration curve for Ethylbenzene

Standard concentrations for toluene were prepared from the stock solution in the concentration range of 10-70ppm (Appendix 1). Table 3.16 and figure 3. 10 represent the calibration data of ethylbenzene.

Table 3.16 **Calibration data for Ethylbenzene standard concentrations and chromatographic peak eights.**

Conc. (ppm)	**Peak Area** (mV s) 3 injections			**Average Peak Area** (mV s)	**Retention Time** (s)
	1	2	3		
10	5.20	4.75	4.98	4.98	12.61
20	10.3	10.7	11.0	10.67	12.61
30	16.8	17.2	17.7	17.23	12.62
40	23.9	22.8	23.3	23.33	12.62
50	29.0	29.7	30.7	29.80	12.63
60	36.8	35.5	35.2	38.20	12.63
70	40.9	44.4	40.1	41.80	12.64

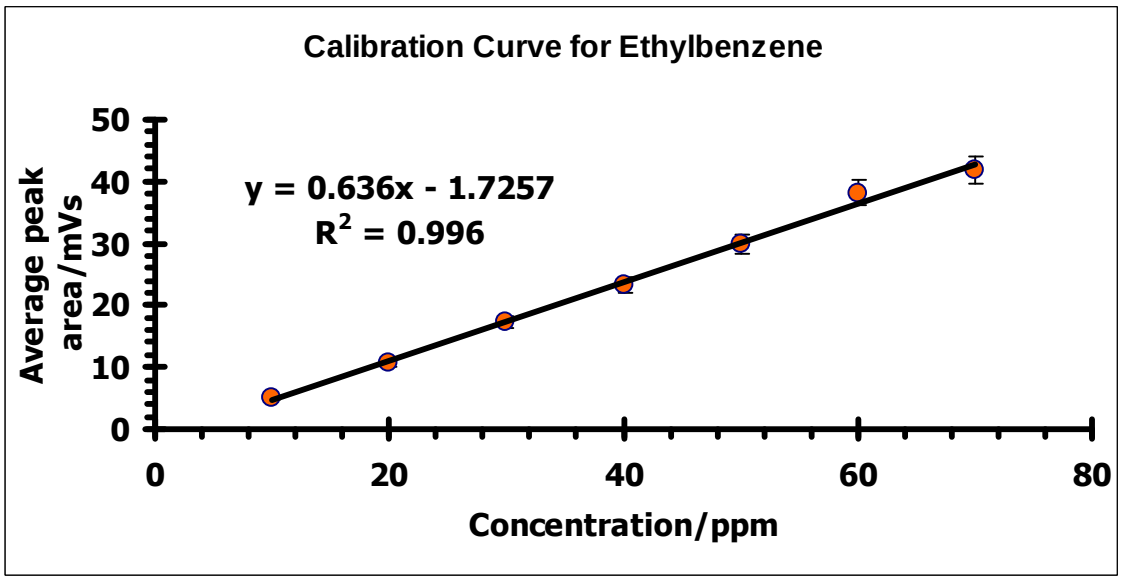


Figure 3.10 **Calibration curve for ethylbenzene for concentration range of 10-70 ppm. Triplicate measurements were taken. R^2 value is 0.99 at 95 % confidence level**

3.5.4 Calibration curve for o-Xylene

Standard concentrations of toluene were similarly prepared as for benzene for concentration range of 10-70ppm (Appendix 1). The calibration curve is shown in figure 3.11

Table 3.17 **Calibration data for o-xylene standard with concentration and chromatographic peak heights**

Conc. (ppm)	**Peak Area** (mV s) 3 injections			**Average Peak Area** (mV s)	**Retention Time** (s)
	1	2	3		
10	5.65	5.75	5.64	5.67	14.22
20	13.0	12.2	12.5	12.57	14.22
30	18.8	18.0	18.0	18.0	14.22
40	24.6	25.8	25.5	25.3	14.22
60	37.7	37.5	39.4	38.2	14.22

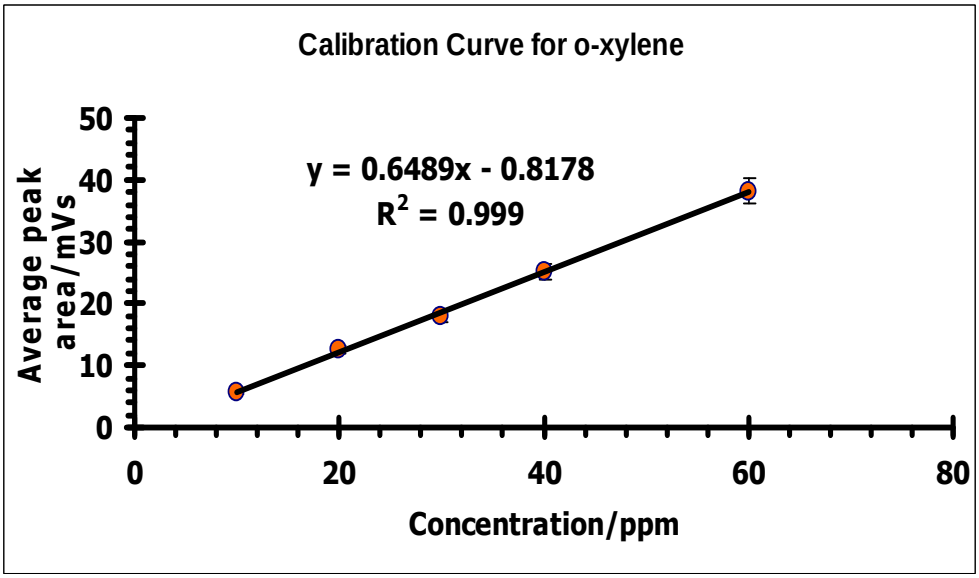


Figure 3.11 **Calibration curve for o-xylene for concentration range of 10-60 ppm. Correlation coefficients (R^2) obtained by linear least-square analysis is 0.99. Error bars designates 95 % confidence level on triplicate measurements**

3.5.5 Calibration curves for Liquid Hydrocarbons

Stock solution of n-pentadecane and some selected hydrocarbons (decane, undecane and tetradecane) were prepared by accurately measuring 100µl into a 10mL volumetric flask and carefully making it up to the mark by heptane. The concentration of the stock solution prepared for n-pentadecane was 7.69 x 10^{-3} gcm^{-3}) (Appendix 1).

Standard solutions were prepared by diluting known volumes of the stock. Six standard solutions were prepared by measuring 100µl, 200 µl, 300 µl, 400 µl, 500 µl and 600 µl into a 10mL volumetric flask and diluting it to the mark by heptane.

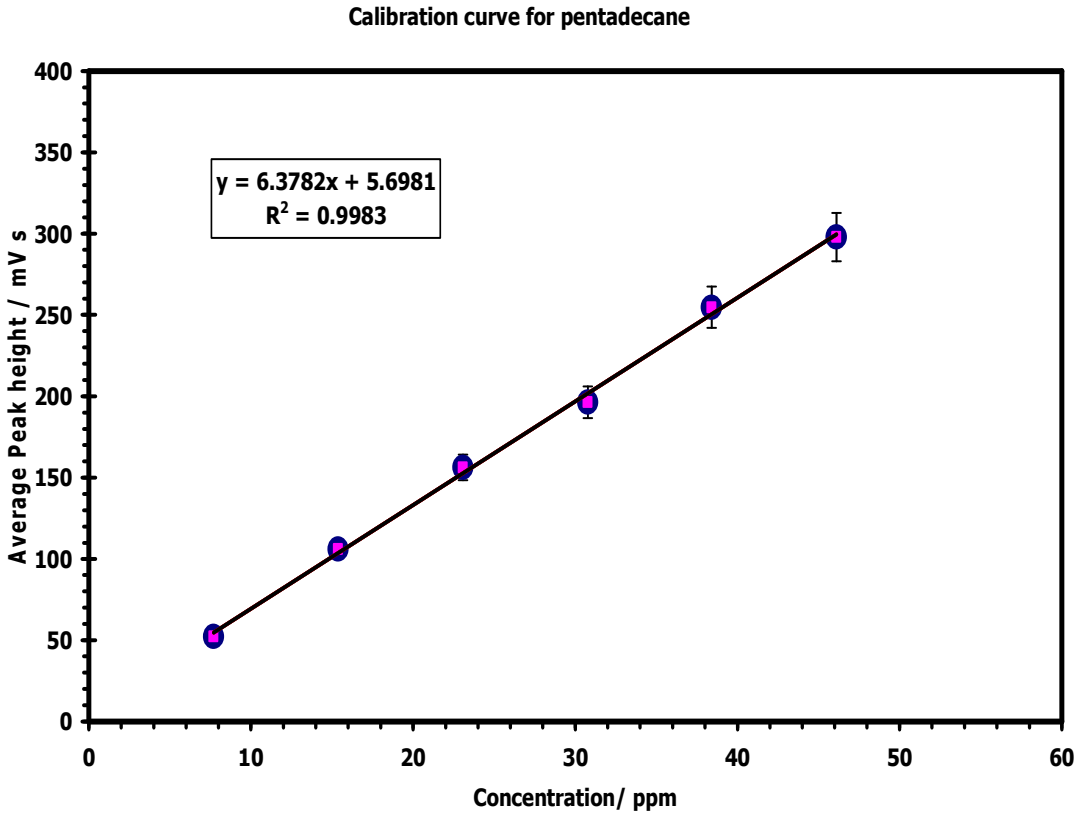


Figure 3.12 **Calibration curve for n-pentadecane at the concentration range of 5-50 ppm. Triplicate measurements were taken. R^2 value is 0.99 obtained by least-square measurement. Error bars were obtained 95 % confidence level.**

Calibration curves for decane (C10), undecane (C11) and tetradecane (C14) are shown in figures 3.13-3.15

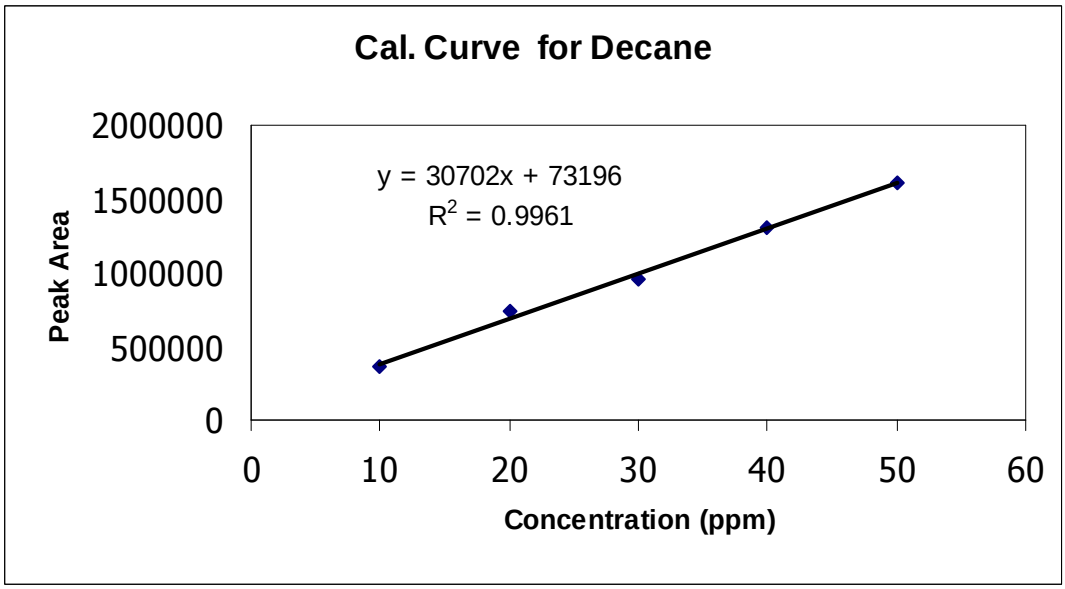


Figure 3.13 **Calibration curve for n- decane. Triplicate measurements were carried out on linear least-square regression analysis. Correlation coefficient (R^2) is 0.99.**

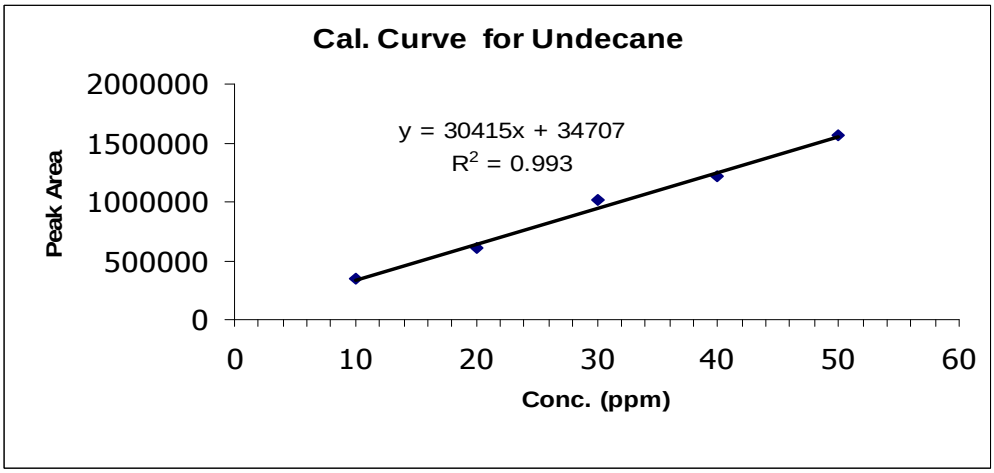


Figure 3.14 **Calibration curve for n-undecane for a concentration span of 10-50ppm. R^2 value was 0.99 obtained from least- square value.**

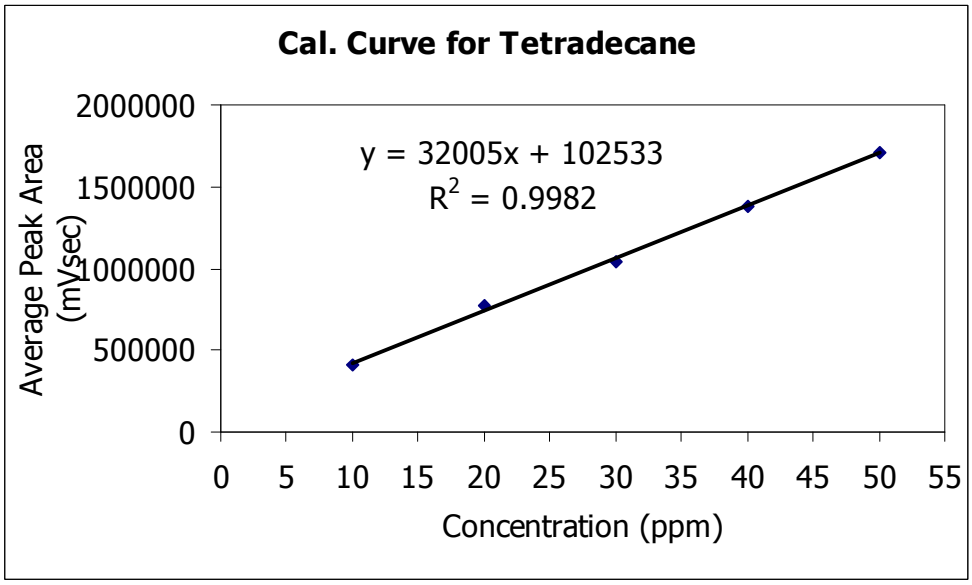


Figure 3.15 **Calibration Curves for n-tetradecane over a concentration range of 10-50 ppm obtained by linear least-square regression analysis. Correlation coefficient (R^2) is 0.99 obtained by Linear least-square. Triplicate measurements were used.**

3.6 Discussion

Tables 3.14-3.17 showed the standard concentrations of each of the standards with their average peak area over a concentration range of 10-70ppm except benzene 10-100 ppm. It is apparent from the data that the peak area increased as the concentration of the standard increased during the analysis.

Figures 3.8 to 3.11 demonstrated the calibration curves for the tested analytes. The calibration linear graphs have straight-line equations with correlation coefficients (R^2) values 0.9999, 0.9872, 0.9998, and 0.9995 respectively for benzene, toluene, ethylbenzene and o-xylene.

The % RSD values for all the calibration curves were less than 6.0 % with standard deviation between 1.5 and 2.5 with reproducibly accepted 95 % confidence level (95 % CL).

The chromatograms indicated minimum background noise with fine peak shapes void of fronting and tailing.

However, the GC-FID method could not detect concentrations lower than 5 ppm for benzene, ethylbenzene and o-xylene as well as 10 ppm for toluene. The precision of the method was satisfactory.

In Figure 3.14 and 3.15, the straight line equation and correlation coefficient for the data is shown for n-pentadecane, decane, undecane and tetradecane. The linearity of the curve has correlation coefficient (R^2) value of 0.99 and %RSD values less than 10% at 95% CL.

3.7 Conclusion

The instrument was calibrated with the concentration range of 10-100 ppm for the analytes-BTEX and n-Pentadecane. The low concentration range was necessary so that such levels of these contaminants will be detected in the sample extract in Chapter 4. However, minimum detection limit for the instruments was established at 10 ppm for toluene, while benzene, ethylbezene, and o-xylene recorded 5 ppm. The calibration linearity was good and the standard deviation and % RSD were satisfactorily low at 95% confidence level. Decane, Undecane, Tetradecane and Pentadecane produced a linear curve with R^2 at 0.99.

All standards were carefully prepared in the laboratory at room temperature similar to the sample ambient temperature.

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CHAPTER 4

DEVELOPMENT OF METHODS FOR THE ANALYSIS OF PETROLEUM CONTAMINATED SOILS

4.1 Introduction

This chapter describes the development, optimization and analysis of extracts from petroleum-contaminated soil samples using Gas Chromatography fitted with a flame ionization detector (GC-FID). This study sought to establish the concentration of the contaminants, assess the penetration and migration of C10-C26 and C26-C34 hydrocarbons through the soil layers and apply cluster observation analysis to characterize chemically similar hydrocarbons and to determine the spatial distribution of these compounds over the contaminated area. This information would be usefully employed in actions that will combat the contaminants through bioremediation process.

4.2 Sampling

Sampling is considered a vital and one of the most crucial steps in the procedure of analysis of organic pollutants in soils and sediments of our environment [1, 2]. A sample is a portion of material selected from a large quantity of material [3, 4]. A sample simply is an informative representative of a population; therefore, it is worthless undertaking the analysis of incorrectly sampled parameters. Sampling activities contribute to a great extent the largest

amount of errors in the entire analysis. The magnitude of this error has been assessed [5] and it is attributed to sampling, transportation, preservation (storage), sample preparation and analysis.

Results generated from various types of environmental analysis have been widely used in academic studies, forensic, agricultural, health assessments to mention but a few. The significant importance of sampling to the overall analytical processes and the error accompanied during sampling is emphasized. Based on this, samples were collected in such a manner to best characterize the extent of contamination of the specific soil. Grab samples (from one location) were collected from different depths with care to minimize the loss of volatiles and contamination with chemicals during handling [6].

The experimental procedure could be summarized in figure 4.1 and terminates in Laboratory analysis.

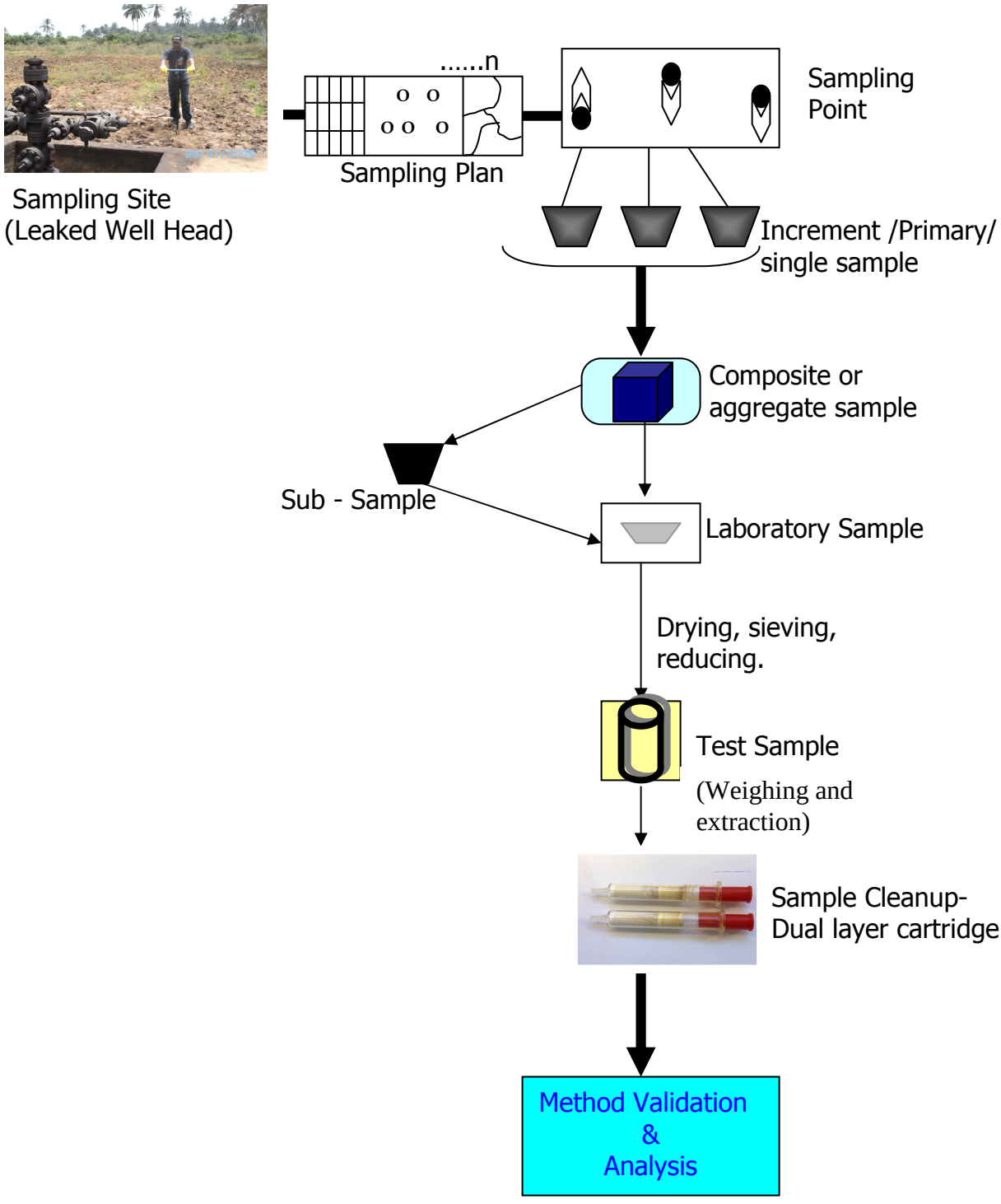


Figure 4. 1 Sampling Operations

4.2.1 Location of Site

A sampling site was located in Ikot Ada Udo, Ikot Abasi in Akwa Ibom State, South-South Niger Delta, Nigeria. At this site, soil and water have been constantly subjected to petroleum spillages and crude oil leakages from a Shell marginal oil pipeline called "Ibibio I" - a Well head established in 1954 as shown in (Figure 1.2, page 24) of chapter one.

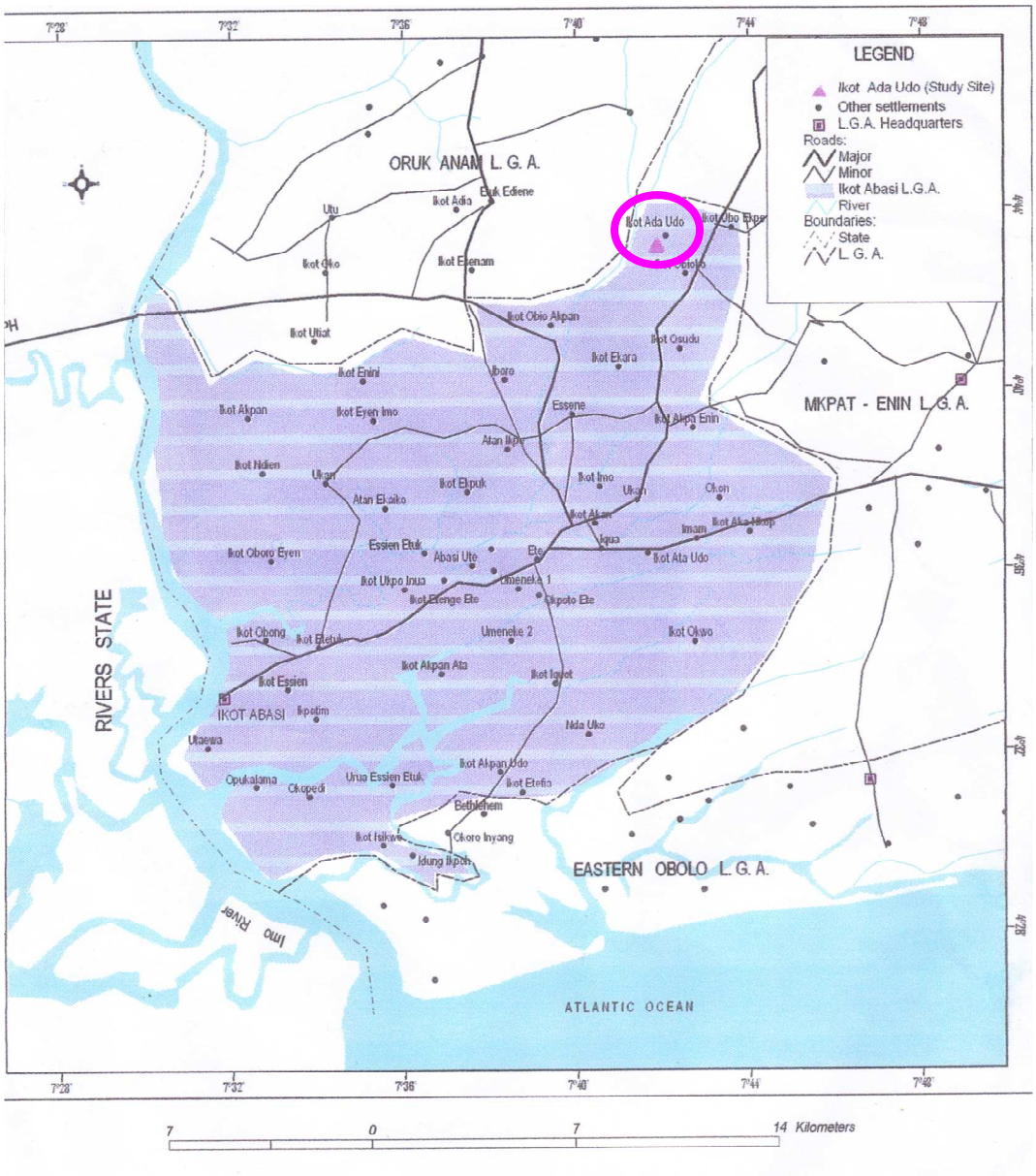


Figure 4.2 Ikot Abasi Local Government Area of Akwa Ibom State Nigeria, showing study site (Ikot Ada Udo) with a pink triangle in pink circle

Prior to the spillage, the site was a good arable farmland where cassava and other agricultural products were grown. This heavily crude oil affected area covered about 200 X 300 m^2. The sampling area and sampling points are shown in Figure 4.3. All sampling points were measured in metres from the well head as a reference point and the soils represented three distinct matrices (loam, clay and sand). The sampling points located on the map were done by sampling.

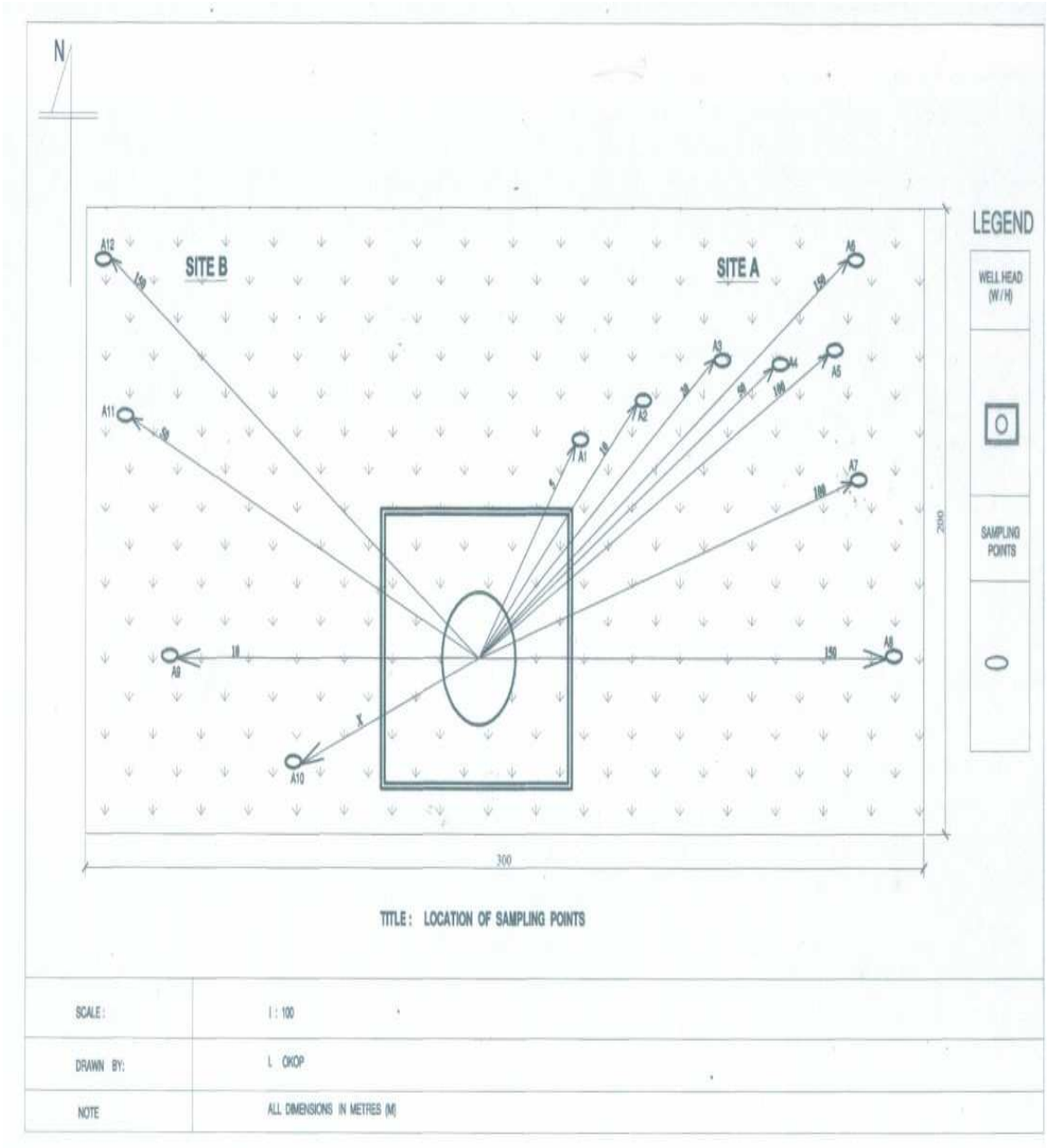


Figure 4.3. Sampling points randomly taken with reference from the Well Head. Measurements in metres (m).

4.2.2 Site investigation

An initial survey was carried out on the site. During this exercise, two personnel accompanied me from Akwa Ibom State Ministry of Environment and another from the Ministry of Petroleum Resources principally to assist and introduce me to the village chief and vigilante groups. The on-site visit was to gather much information about the sampling area and to establish any possible source of obstacle that may arise during sampling. It also enabled me to determine the soil type, the terrain and the feasibility of using the hand soil auger for sampling as well as recognising the correct first aid kit and personal protection equipment (PPE) to take with me. This was done to ensure that problems during sampling would be minimized.

4.2.3 Sample Collection and Number

Soil from various depths on the sample locations were selected to represent areas of contamination resulting from the recent petroleum pipe line spillages. A hand soil auger (Nickel-plated carbon steel, 3" diameter), figure 4.4 was used to collect soil samples from the site by taking about 6-10 auger borings at random grid at sampling points to depths of 0-15 cm at the surface soil , 15-30 cm at middle (sub-surface) and bottom layer of 30 to 60 cm.

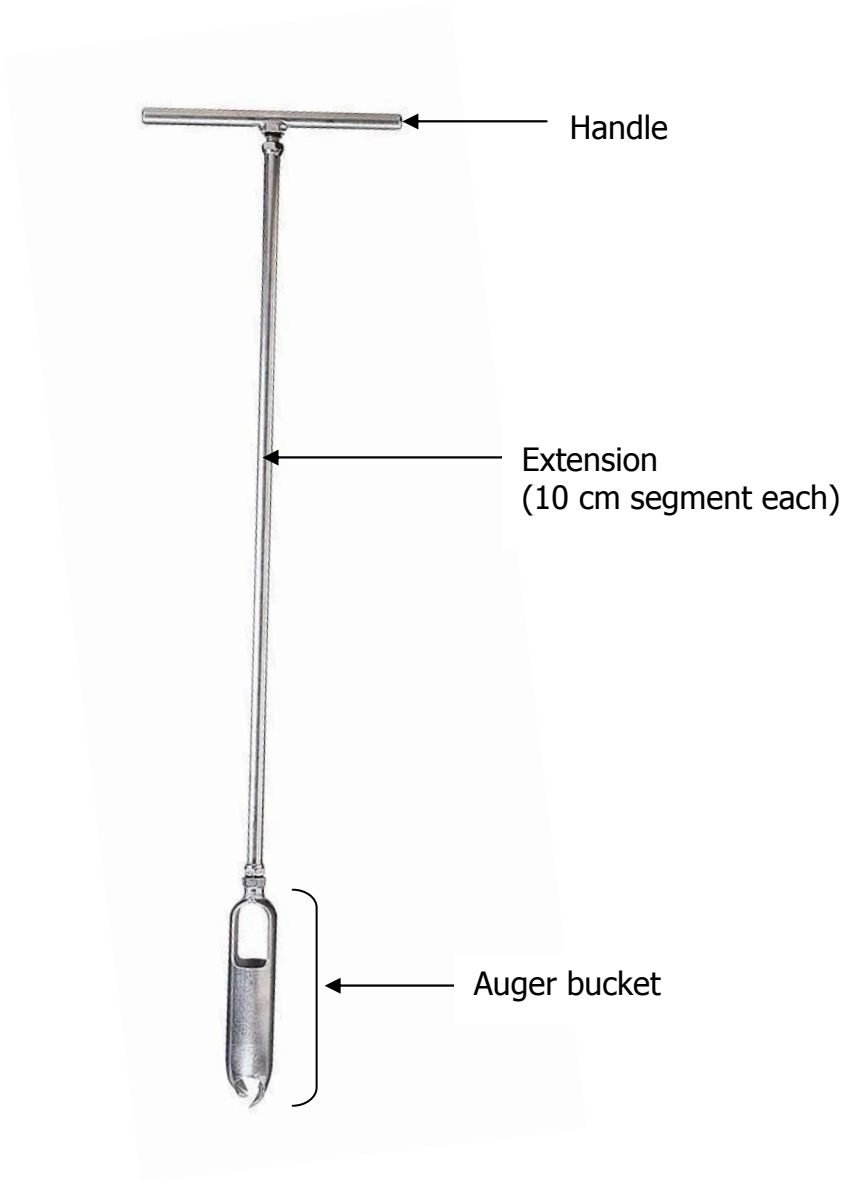


Figure 4.4 Stainless steel hand auger used for the sampling.

Three sub-samples were collected at each sampling point of designated depths. Representative soil samples from the auger were taken into fresh polythene bags with seal and further placed in a pre-cleaned glass bottle with a clean Teflon-lined lid, figure 4.6.

Approximately 500 g of soil was collected at each sample depth by drilling and advancing the desired distance into the soil as auger is withdrawn from the hole and soil removed and the process continued till required depth.

Four control site samples (duplicate sample blanks) were obtained to determine the background levels of petroleum hydrocarbons in the clean soil for comparison with the contaminated soil. Two out of four control site samples were obtained 20 m and 100 m respectively adjacent to the spilled site and possessed common soil characteristics with the contaminated site. The other two control samples were taken from a geographically similar uncontaminated area located about 2-5 kilometres away from the spilled arena.

Figure 4.5 Photographs showing (A) Researcher explaining a point with village representative on site (B) Researcher sampling from least contaminated points.

All four control samples were taken same day prior to actual field samples. In the spilled site, grab samples were taken from least contaminated to most contaminated points (see figure 4.5b). The auger was cleaned with water and rinsed with methanol after each sampling point.

4.2.4 Sampling Equipment

Auger boring often provides the simplest method [7] for soil investigation. Aside From the hand auger, other sampling equipment was employed in the sample process. The list is not exhaustive but includes the following: Field notebook and data sheets, GPS data logger and receiver, zip type plastic bags, sample labels, 250 mL and 500mL sample bottles, cooler and ice, spade and scoop, glass container with seals, measuring tape, Safety boots and glasses, protective nitrile gloves, two internal standards and first aid kit.

4.2.5 Sample Containers and storage.

Samples were collected into zip type plastic bags and placed in a 1 L wide mouth glass jar with Teflon lined cap and seal as shown in figure 4.6. The sample containers were tightly sealed as soon as samples were grabbed then cooled in ice cooler to the laboratory for refrigeration until analysis.

Figure 4.6 Soil samples sealed in a zip type plastic bag and put into a a wide mouth jar with Teflon seal and clip.

All samples were carefully labelled during sampling and were separated from other sampling points during storage. Individual sample was prepared and separately stored in sealed bags into appropriate containers awaiting weighing and extraction (See Appendix II A).

4.2.6 Sample Procedure and Labelling

This is an important exercise just as protecting the integrity of the samples. An appropriate label with the sample details was attached to each bottle. Labelling on sample bottles included the date and time of sampling, sample location, sample number, temperature of sample, ambient temperature, sampling coordinates and distance of samples from the reference point – the well head.

The following details were recorded on a sample data sheet during sampling:

date, location number, sample number, distance from the well head, direction coordinates, problems/remarks, soil temperature, and ambient temperature.

The auger was twisted clockwise until the bucket was full, it was then withdrawn and the soil removed. This process was continued until each sampling point was reached. Graduation marks on the auger made it possible for easy identification of the required depths.

The table of sample identity and description is presented on table 4.1.

Table 4.1 Sample Description Table

Hole	Sample ID	Description	No. of Samples	Depth (cm)	Position Coordinates North East
1	A1	5 metres (m) from Well head	3	0-15 cm 15-30 cm 30-60 cm	4° 41′ 49.4″ & 7° 41' 09.8"
2	A2	10 m from Well head	2	0-15 cm 15-30 cm	4° 41′ 50.5″ & 7° 41' 09.12"
3	A3	20 m from Well head	3	0-15 cm 15-30 cm 30-60 cm	4°41′ 49.3″ & 7° 41' 11.4"
4	A4	50 m from RP	3	0-15 cm 15-30 cm 30-60 cm	4° 41' 48.6" & 7° 41' 10.1"
5	A5	100 m from RP	3	0-15 cm 15-30 cm 30-60 cm	4°41' 47.2" & 7° 41' 09.9"
6	A6	150 m from RP	3	0-15 cm 15-30 cm 30-60 cm	4°41' 48.8" & 7°41' 09.1"
7	A7	100 m from RP	3	0-15 cm 15-30 cm 30-60 cm	4° 41' 49.7" & 7° 41' 09.3"
8	A8	150 m from RP	3	0-15 cm 15-30 cm 30-60 cm	4° 41' 47.7" & 7° 41' 07.6"
9	A9	10 m from RP	3	0-15 cm 15-30 cm 30-60 cm	4° 41' 50.3" & 7° 41' 10.6"
10	* A10	Well Head, (WH) - (Spillage and Reference Point. RP)	3	0-15 cm 15-30 cm 30-60 cm	4° 41' 49.8" & 7° 41' 10.4"
11	A11	50 m from RP	3	0-15 cm 15-30 cm 30-60cm	4° 41' 48.4" & 7° 41' 09.7"
12	A12	150 m from RP	3	0-15 cm 15-30 cm 30-60cm	4° 41' 49" & 7° 41' 09.5"
13	A12X	NA	3	Same as above	-
14	AX13	NA	3	Same as above	-
15	AX1	NA	3	Same as above	-
16	AX2	NA	3	Same as above	-

In table 4.1, samples A1 to A12 were collected at actual contaminated sampling points on site while A12X to AX2 are the control samples taken within the vicinity.

4.2.7 Preservation and Transportation

All samples were placed in icebox in a car after completion of sampling at site and taken to the airport for shipment. The entire sampling exercise was carried out in one task-full day. The minimum temperature recorded for the soils sampled was 29°C and the maximum recorded was 38.6°C while the average was about 35°C. The average ambient temperature was 28°C.

The samples were shipped the same day by air to United Kingdom for analysis by the chemical shipping agent in Nigeria with full special shipping procedures for transporting and handling the samples [8]. In two days the samples were received and stored in the laboratory fridge at 4°C until analysis. The temperature of the samples on arrival in the laboratory was between 12-15 °C.

4.2.8 Problems Experienced During Sampling

The first problem occurred when a large stone was encountered during sampling of point or hole A2 after the 30 cm depth, thereby posing an obstacle to sample the last point at 60 cm depth. This may be concrete buried during the installation of the Ibibio 1 Wellhead. As a result, two samples were taken at the 0- 15 cm top soil and 15-30 cm middle soil and the third that should have been taken at greater depth was discarded due to improper sampling.

Other problems encountered during sampling were the excessive sunshine and high temperature, which inherently led to inadvertent loss of volatiles, biodegradation, oxidation and reduction as [9] shows that high temperature would accelerate these processes. Low soil moisture content and high soil porosity will enhance volatility, which was not specified in ASTM method.

The problem of militancy in the politically volatile Niger Delta also caused problems during the period of sampling. Hence the sampling process had to be completed within a single day because access to the site was denied.

4.3 Extraction

4.3.1 Introduction

A typical Soxhlet apparatus is shown in figure 4.7 and the laboratory set up is displayed in Appendix II (E-F).

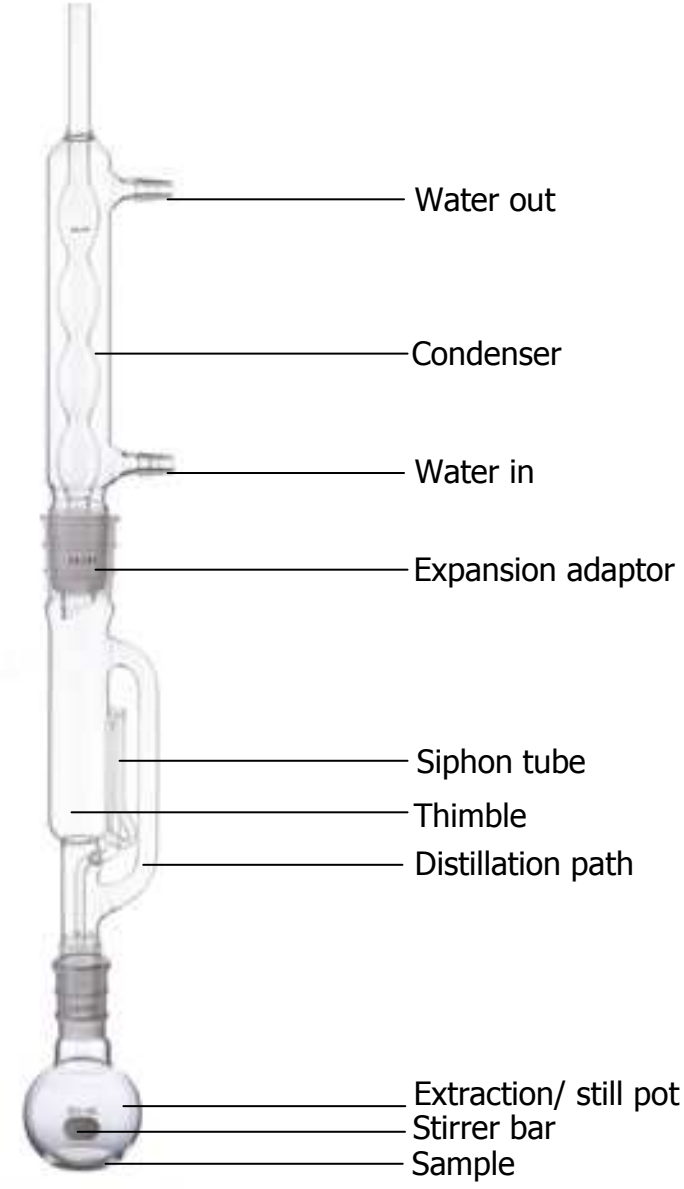


Figure 4.7 A display of a typical Soxhlet Apparatus

Extraction of petroleum contaminants from the soil had to take place prior analytical determination. Soxhlet extraction using a Brinkmann Büchi 461 automated extraction apparatus was used in this work.

Soxhlet extraction is a U. S. EPA [10] and ASTM [11] approved method for semivolatile and non-volatile organic contaminants from solid materials such as soil. All samples were extracted using the Soxhlet extraction procedures as outlined in U.S. EPA method 3540 [10] and ASTM method D5369 [11] with slight modifications in the solvent choice and volume, extraction time and size of extraction flasks. Soxhlet extraction really ensures intimate contact of the sample matrix with the extraction solvent and a reasonably large amount of 3-20g could be used to allow quantitative extraction. Soxhlet technique is usually the adopted reference and most often used method for a long time. It has been proposed by many agencies [12, 13] as a method of choice for extraction of non-polar organic contaminants.

Easily assembled, non-instrument apparatus, relatively inexpensive, good extraction reproducibility, capable of 3-6 simultaneous extractions (see appendix II F, G, H) and solvent intimate mix with the sample matrix are but inexhaustive advantages of Soxhlet extraction techniques. Like every other technique, it has its drawbacks which include long extraction period and use of appreciable volume of solvent. Other methods may not be labour intensive but due to their cost may not be accessible to every laboratory.

4.3.2 Sample Preparation

Large rocks, pieces of pebbles, plant roots and sticks were removed from the samples. They were air-dried at room temperature similar to the sampling milieu. The samples were spiked as they were collected on field with 1000 ppm of two hydrocarbon internal standards, tetradecane ($C_{14}H_{30}$) and pentadecane ($C_{15}H_{32}$) before extraction.

All the samples were tightly sealed with minimized headspace in a Teflon zip polythene double bag and placed in a tightly closed jar to ensure that the loss

of volatiles is minimized as seen in figure 4.6 above. The samples were crushed and sieved to fine size and stored in a well-corked small container for extraction to proceed as shown in Appendix II A.

4.3.3 Sample Extraction and Clean-up

Soil samples were weighed using a standard electronic top-loading 'Mettler' analytical balance. The Büchi extraction apparatus consisted of the Soxhlet extractors, rotary evaporator and the water bath. HPLC grade dichloromethane (DCM) was used as the extraction solvent. Its chemical safety data is provided in appendix II (I). Other solvents used for comparison were hexane, toluene and acetone or mixture. Other materials used were methanol, beakers, measuring cylinders, spatula, pipettes, glass wool, anhydrous sodium sulphate, Cellulose thimble, 4 mL GC vials and labels and stickers. Many solvents are recommended for hydrocarbon extraction, but the efficiency of these solvents for the extraction of hydrocarbons was not known exactly. Therefore, three different solvents were chosen viz; dichloromethane, hexane and toluene. It was necessary to establish which solvent was the most appropriate for the extraction process adopted. The Büchi apparatus, being a readily available, enhanced and improved method of Soxhlet extraction was chosen for this wok.

Previous studies [14, 15] have reported the determination of the best extraction solvent and the optimum time needed to extract hydrocarbons by Büchi apparatus. Further confirmation was required as the extraction time was restricted to 2- 5 hours. This research showed that dichloromethane (DCM) proved to be the most consistent and efficient solvent for reproducible extraction of the soils over the time interval considered. The other solvents were either inconsistent or slow to initiate extraction while DCM will have already started the extraction immediately (refer to Appendix II (G & H). DCM was also considered the best choice of solvent for this work over toluene and hexane since its retention time did not interfere with the BTEX reference standard retention window, alkane mix standards (prepared in hexane) and the GRO (Gasoline Range Organics) in samples which fall in this retention time window.

Approximately 10 g of homogenized dry soil was weighed into a Whatman fat-free extraction (filter) thimble (26 X 60 mm) and Soxhlet extraction commenced [16, 17], as a continuous extractor for organic matter. 5 g of anhydrous sodium sulphate was mixed with the soil to remove water content and thereby improve the Soxhlet Extraction to the organic phase.

Before the extraction started, the soils were homogenized using mortar and pestle to obtain finer texture and to remove sticks, pebbles and rock particles.

The soil samples and blank were extracted with 100 mL DCM for optimum time of 2 hours using a glass Soxhlet extraction apparatus with a 250 mL collection flask. An automated extraction apparatus (AEA), (Brinkmann Büchi 461) fitted with water bath set at ≤ 40°C was used with slight modifications. Soxhlet thimbles were dried at 105°C overnight to constant weight, to keep out moisture and any volatiles then cool to room temperature in desiccators and weighed to the nearest 0.1mg. Weights of samples, blanks and silver sand used were measured to four places of decimal (refer Appendix II B, C & D).

The Soxhlet extraction system was tested for proof of possible contamination by adopting the following steps:

(i) 10g of Silver sand (Fisher Scientific), used as clean sample without hydrocarbon contamination was carefully weighed and placed inside the thimble which was loaded into the main chamber of the Soxhlet extractor. A plug of glass wool was placed on top of the silver sand to prevent sample splash and loss during extraction. The sand, thimble with the glass wool was weighed before the extraction. DCM was placed in the round bottomed extraction flask, heated to reflux via a fitted condenser and extraction commenced. The choice of DCM as a solvent supersedes the use of any hydrocarbon solvent, as this may likely contribute its peak in the analysis. DCM was also tested with other solvents and it proved to be efficient and consistent. After extraction, the extract in the flask was first concentrated to about 25 mL by rotary evaporator set at 40°C±1. The flask was allowed to cool and weighed to the nearest 0.1mg. The extract was pre-cleaned and kept for GC analysis.

(ii) Extraction was carried out with DCM on the weighed, dry fat-free cellulose extraction filter thimble without the sample. The extract (solvent) was concentrated via the rotary evaporator to 25 mL. Analysis of the aliquots in a 2 mL vial was done using the GC-FID.

(iii) Three selected samples were also spiked with tetradecane and pentadecane, weighed and extracted in similar condition using DCM at the optimum extraction time established in section 4.3 4. Their average percentage recovery was calculated to be ≥ 80%. These three pre-extraction steps proved that the extraction apparatus was free of possible contamination.

4.3.4 Sample Clean-up process

Each of the sample extracts were cleaned to remove moisture, polar hydrocarbons, colour interferences and any impurities before subjecting them to GC column analysis. This was achieved by filtering the extract under applied pressure through dual layer 6 mL glass Florisil®/Na_2SO_4 SPE Tube 2g/2g, 40080-1 ea-f supplied by Fluka Analytical, Sigma Aldrich Chemie GmbH, Switzerland. (refer to figure 4.8).

The kits have a tube with 2g Na_2SO_4 (99.99%) purity and density of 2.68g/mL) at upper layer and 2g Florisil® (magnesium silicate) in the lower layer, separated and packed with 20 µm PTFE frits. The upper Na_2SO_4 layer aids in removing aqueous sample residues that may hinder Florisil® performance and /or subsequent GC analysis. The efficiency of clean-up by the ratio of stearyl stearate peak area determination is< 10% (treated/untreated-EN14039), < 5% (ISO 16703) and the recovery rate of the mineral oil standard solution is > 90 % (EN 14039), > 80% (ISO 16703). The clean-up SPE Tube is suitable for determination of the hydrocarbon oil index in soil, waste (liquid and solid), water (surface, waste and sewage treatment plants) by GC-FID analysis according to ISO 16703 (Soil), EN 14039 (waste) and EN ISO 9377-2 (water).

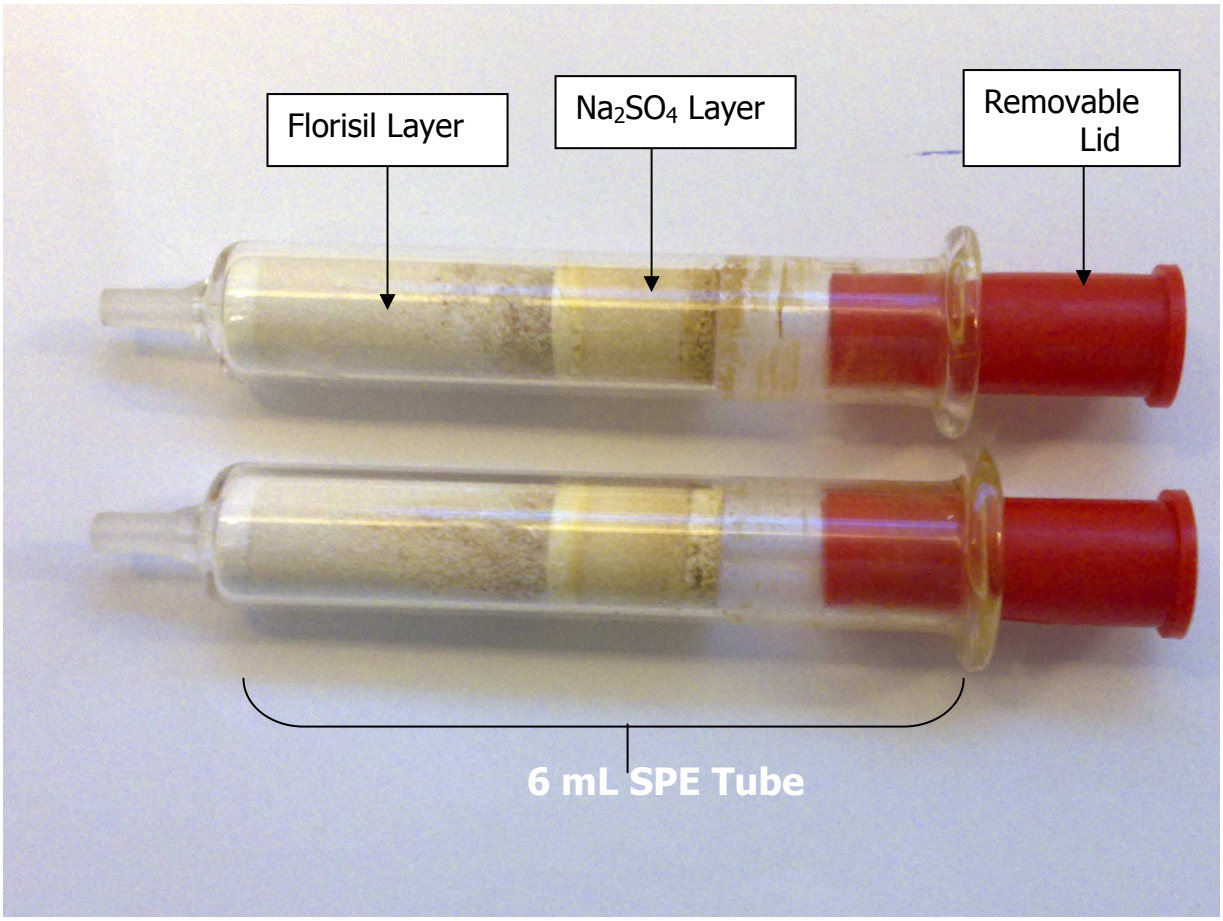


Figure 4.8 A pictorial representation of used dual layer Florisil®/Na_2SO_4 tube for sample clean-up aimed at removing moisture, polar hydrocarbons, colour interferences and impurities.

The clean-up procedure effectively removed hydrocarbons of natural origin and did not have any significant effect on the amounts of petroleum hydrocarbons present.

4.3.5 Comparison of Optimum Extraction Time

Sample preparation could become a cumbersome task especially when considering large number of samples. It therefore became imperative to ensure that the extraction time was optimally established to eschew unquantifiable waste of time. Experiment was carried out to verify what time would be used to

extract a known amount of soil samples of this characteristic geographical region based on the investigation done by [14, 15] using five and two hours limit respectively.

Sample A3 (3 sets) was randomly chosen for this process. About 10g of the sample was weighed as previously described and extraction commenced with all the parameters kept constant except time of analysis. The extraction was carried out from 1-7 hours at repeated batches. At the completion of the set time, the solvent was evaporated using preset hot water bath. After cooling in a desiccator, the extracts were weighed accurately to 0.1g. The percentage of the residue was calculated based on the mass of the residue and the total amount of soil used for extraction. The percentage yield of the extracts was calculated from the following relationship.

$$\text{Percentage Yield} = \frac{\text{Mass of extract}}{\text{Mass of sample}} \text{ X } 100$$

The result gave two-hour optimum extraction time for the tested representative sample. The result and table is shown in chapter 5, table 5.2 and figure 5.1 of pages 168 & 169).

4.4 Experimental

Forty-seven (47) soil samples collected from an oil spill location in Akwa Ibom State, South-South of Niger Delta, Nigeria, were stored, prepared and analyzed using Gas Chromatography (GC). GC seems to be a good alternative to measure total petroleum hydrocarbon and individual products present in a sample while such identification by IR may be impossible. Generally, GC method has almost no restrictions with regard to the extraction solvents. The Organic pollutants, mainly total petroleum hydrocarbon (TPH) were determined using the GC-FID. FID has an advantage over PID because FID can detect wide range of volatile and non-volatile organics even at lower detection limits of sub parts-per million levels.

4.4.1 Gas Chromatography Instrumentation

Chemical analysis for the determination of hydrocarbons in the soil was performed on all the soil samples and standards using a Varian model BV CP – 3800 GC equipped with a split/splitless injection port and Combi PAL auto sampler (figure 4.9). All samples were taken into 2 mL chromatographic vial, injected and separated on a Varian Chrompack capillary column CP 5860 with 95% methyl and 5% phenyl-polysiloxane phase, (oven max tempt 350°C), WCOT fused silica, 30 m x 0.25 mm id x 0.39 mm od and 0.25 µm film thickness with CP-Sil 8 CB low bleeds/MS coating.

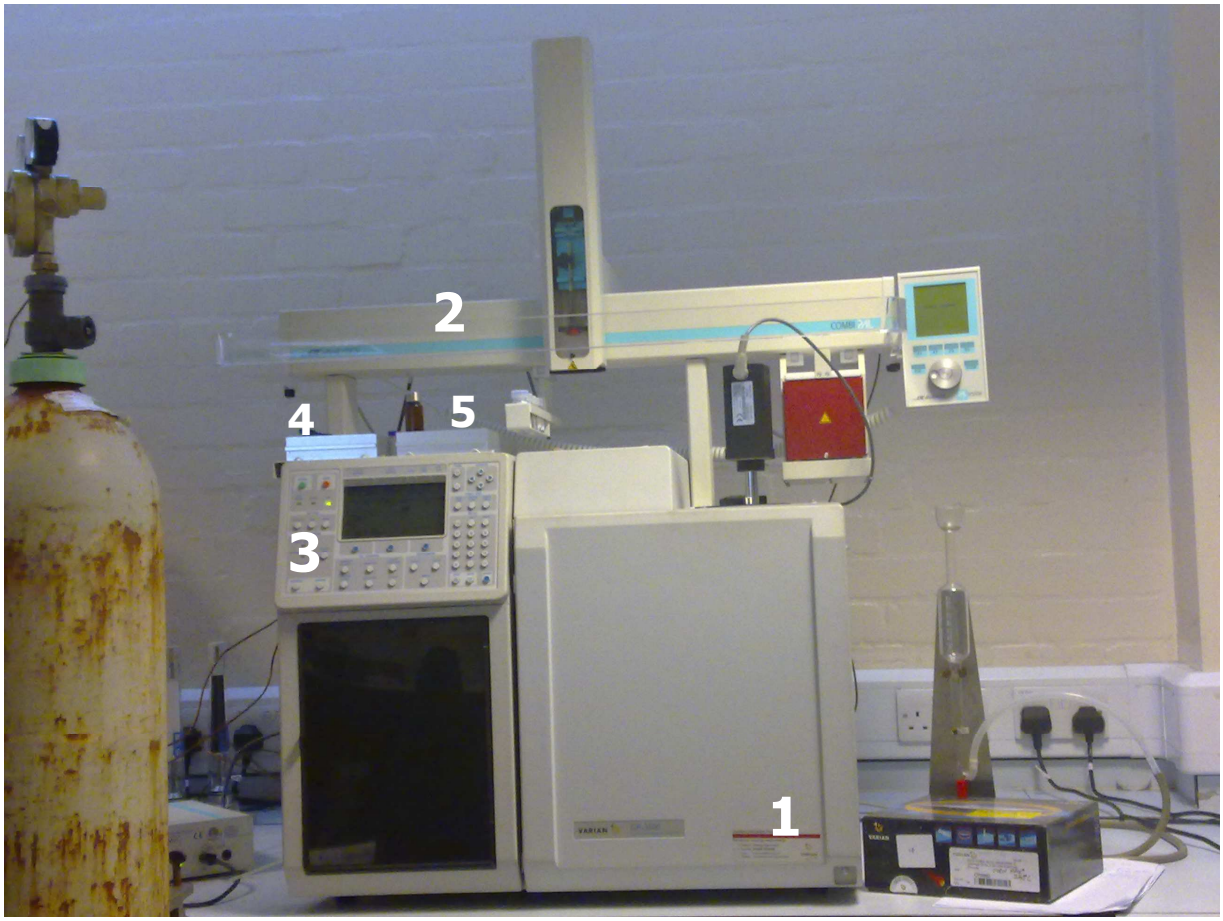


Figure 4.9 A Photograph of GC-FID Instrumentation

1) column oven. 2) Combi Pal auto sampler unit. 3) control panel. 4) first sample tray (for 2mL vials). 5) second sample tray (for 20 ml headspace vials).

Selecting this column was the best choice when developing a method due its high column efficiency, better selectivity, guaranteed retention times and reproducibility. The column has higher polarity long time and suitable for a wide range of applications for aromatic compounds, alcohols, amines, phenols, organic acids, PAHs, vast range of hydrocarbons steroids, sterols, PCBs, sugars and EPA methods. However, the column was not able to attain the maximum temperature specified by the manufacturer probably due to method and instrument efficiency.

4.4.2 Materials

In this work, dichloromethane (99.8%) used as the extracting solvent was supplied by Sigma Aldrich, Steinheim, Germany. Reference standards used were BTEX Mix, catalog No 47993 supplied by Supelco analytical, Bellefonte, PA, USA, 56681 Kit for the chromatographic determination of hydrocarbon content in soil according to DIN ISO 16703 and waste according to EN 14039 comprising of a standard solution for the determination of the retention window (RTW), cat. No 67583, Mineral Oil standard mixture type A and B for DIN EN 14039 and ISO 16703 (cat. No. 69246), Alkane standard mixture for the assay of the system efficiency of GC's (C10-C40) cat. No. 68281, Heptane, Puriss.p.a (cat. No. 51745) and Dual layer Florisil®/Na_2SO_4 SPE Tube 2g/2g/6mL (Cat. No 40080-1ea-F) all supplied by Fluka Analytical, Sigma Aldrich, Switzerland. DRO Mix (Tennessee/Mississippi), catalog No. 31214, Lot No. AO62141 was supplied by Restek, 110 Benner Circle, Bellefonte, PA, USA. Hydrocarbon internal standards C14, C15 and verification standards (C10, C11, C14, C15 and TCD) prepared in the laboratory were all HPLC and analytical reference grades.

4.4.3 Reference Standards

The following reference standards and calibration mixtures were employed for qualitative, quantitative analysis and instrument calibration.

(i) Alkane standard mixture, 50 mgL^{-1} (C10-C40, even carbon numbers) in hexane containing 16 different hydrocarbons.

(ii) Standard solution (C10 & C40) for the determination of the retention time widow (RTW), 0.03 $mLmL^{-1}$ (30ppm) prepared in hexane.

(iii) Mineral Oil Standard mix, 8.00 $mLmL^{-1}$ in hexane.

(iv) BTEX Mix, 2000 μgmL^{-1} of each of the following components in methanol.

(v) DRO Mix, (C10- C25) 1000 μgmL^{-1} each in dichloromethane containing 16 various hydrocarbons.

(vi) TCD –Thermal Conductivity Detector standard (C14, C15 & C16), in methanol and Dodecane.

(vii) Laboratory prepared Hydrocarbon standards in DCM explained in Section 4.4.4 below.

4.4.4 Standards Preparation.

Stock standard solutions were prepared and stored in appropriate containers. Standards derived from the laboratory were decane ($C_{10}H_{22}$), undecane ($C_{11}H_{24}$), tetradecane ($C_{14}H_{30}$), pentadecane ($C_{15}H_{32}$) and tcd (mixture of C14, C15 & C16). 1000 ppm (mgL^{-1}) of each of the standards was prepared from their stock solutions.

50 mgL^{-1} (50 ppm) of alkane standard mix (C10-C40) containing sixteen compounds (even carbon number) was prepared for use in the GC calibration.

03 mL mL^{-1} (30 ppm) of RTW standard was prepared and used for window retention time calibration of the instrument.

1000 μgmL^{-1} of DRO mix (Diesel range organics) containing 16 hydrocarbons was used for the GC calibration.

1000 μgmL^{-1} of BTEX was prepared from its stock of 2000 μgmL^{-1} for GC calibration in the aromatic hydrocarbon range.

All these solutions were prepared and stored in their approved containers in the fridge just before analysis.

4.4.5 GC Method Development and optimization

The process of method development started by imputing the required GC parameters through the Varian Star™ software. Data were automatically stored and retrieved from this software whenever required. Temperature programming (Ramp) was the programme mode. The column was fixed, tested and conditioned according to the manufacturer's guide. When the column conditioning was completed, 1 µL of Varian test mixture chromatogram CP 5860 in cyclohexane was run to determine column quality and the chromatogram was compared with the supplier's chromatogram. The extracting solvent was injected in triplicates and repeated to establish its retention time.

Test sample analysis was carried out to obtain the expected separation and the concomitant retention time.

The GC optimization process progressed with the adjustment of the following GC parameters to achieve better separation with good base line.

- Starting/initial column temperature and hold time was varied between 30°C –250°C and 2-5 minutes respectively.
- The ramp rate was varied from 5 °C to 20 °C per minute
- Split ratio was adjusted from splitless to spilt ratio of 1:25.
- Final column temperature and hold time was continuously adjusted between 250°C – 320°C and 5-25 minutes respectively. The reference standards, (Alkane standard mix & DRO standard mix), BTEX, laboratory standards and some samples were injected continuously to establish the required optimized method.

Method development and optimization proved to be a very taskful exercise until the desired goal was attained. However, persistent adjustment of the method parameters finally yielded the optimized condition of method development based on better separation and good peak response.

Table 4.2 Summary of instrumentation parameters of optimized GC method.

GC-FID Parameters	
Carrier gas	Helium (Linear velocity 26 cm sec^{-1})
Column flow rate	1.0 mL min^{-1}
Sample injection volume	1 µl
Start/Initial column temperature	30°C
Initial Holding time	3 min
Temperature ramp	8 °C min^{-1}
Final column temperature	320 °C
Final Holding time	15 min
Total time of analysis	51.75 min
Split ratio	1:25
Rear Injector (**Varian1177)** temp.	300 °C
Detector (FID) temperature	300°C
Hydrogen flow rate	30 ml min^{-1}
Air flow rate	300 ml min^{-1}

Sensitivity of the GC-FID is 15 picoCoulomb/nanogram Dodecane.

The instrument was now programmed under the established optimum method. Therefore maximum sensitivity, better peak shapes and reduced retention time

(run time) were obtained and applied as the optimized method. The optimized GC-FID method used in the determination of the petroleum-contaminated soil is shown in table 4.2 with slight modification but similar to that in U.S.EPA [18] and API [19, 20, 21] method development guidelines.

Calibration of the instrument under this condition was done by injecting the blanks, reference standards, tcd, lab standards and samples in triplicate and their retention times were programmed and stored in the Star^{TM} software methods file.

4.5 Analysis

The blanks (solvent and thimble blank extract) were run several times to ensure that the column is free from any possible contaminant and also to test the extraction apparatus (figure 4.10).

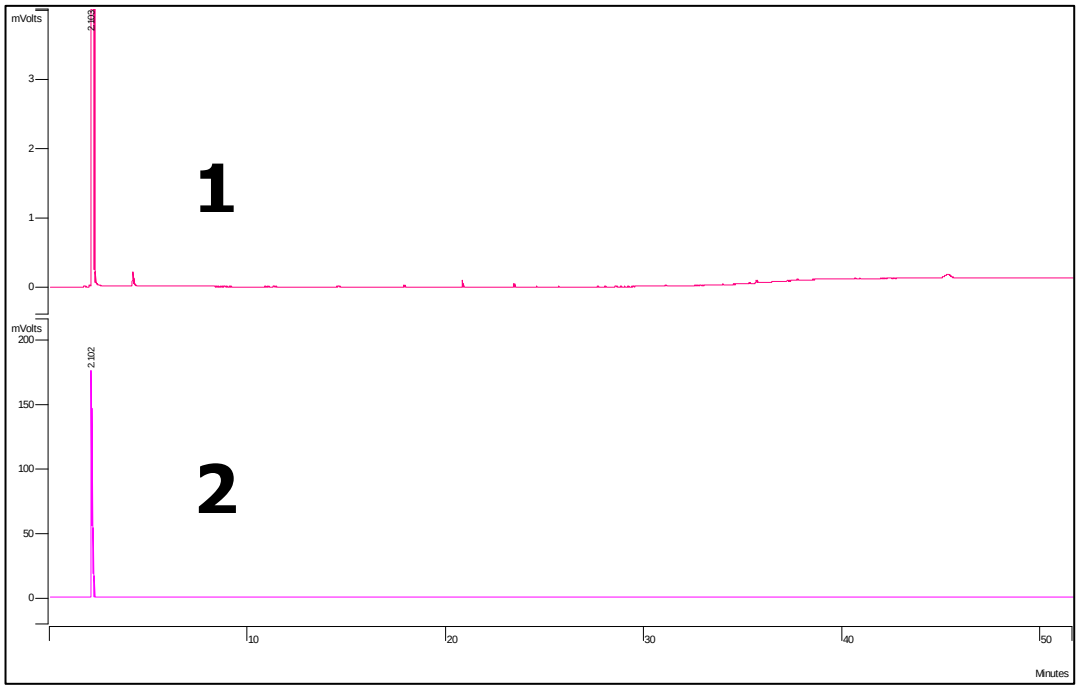
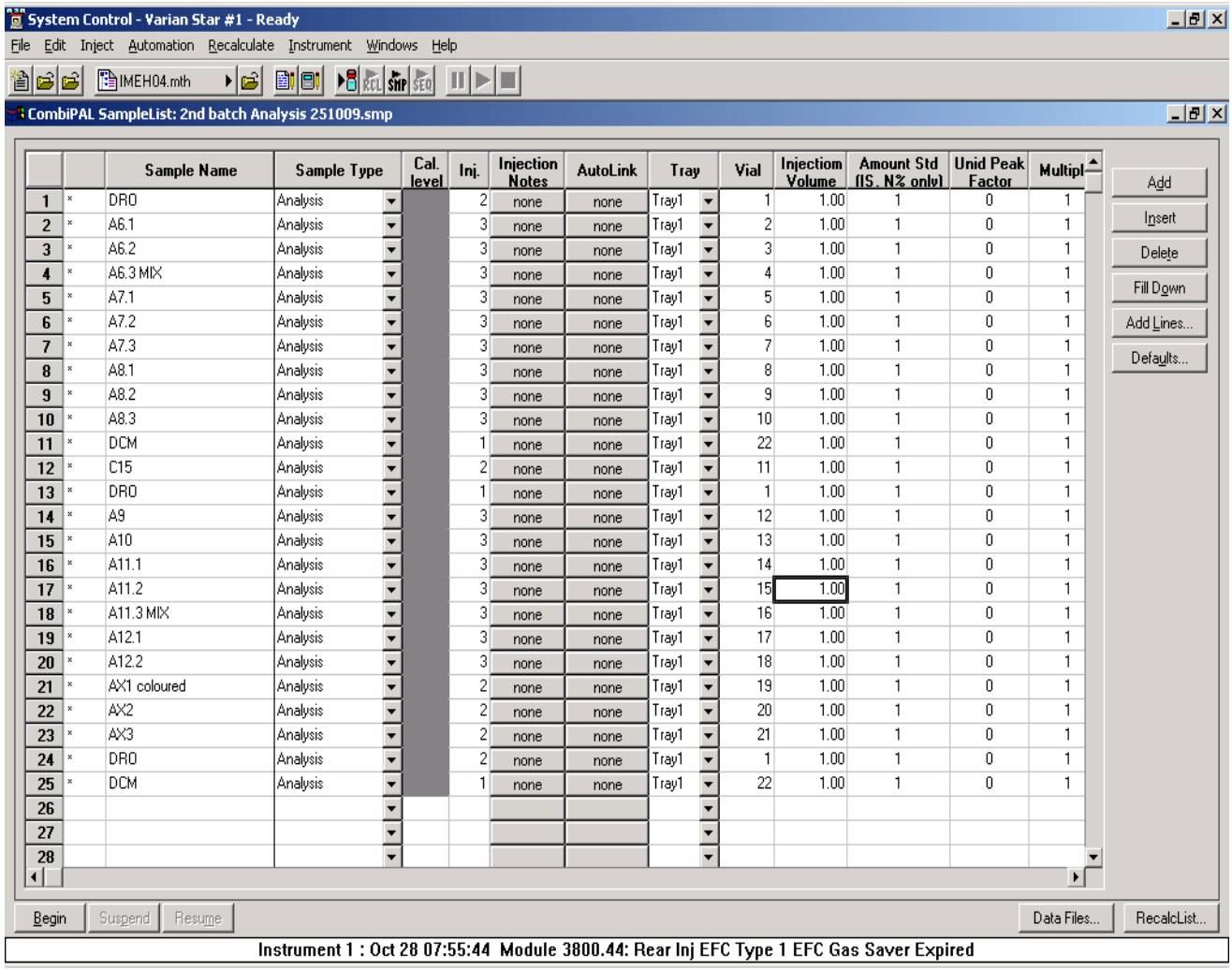


Figure 4.10 **1) Chromatogram of neat Solvent (DCM).**
2) Chromatogram of thimble blank (extract of solvent + thimble & no sample)
The chromatograms indicated the retention peaks of the extracting solvent (2.1 min). This was a step to test that the extraction apparatus was not contaminated originally. The chromatogram is linear and has no indication suspicious of peaks.

Automated sample lists were prepared using the star^{TM} software and stored in the computer (See sample list in figure 4.11)

This alkane hydrocarbon standard was analysed concurrently with the sample A3 extract chosen during the optimization process. The aim was to produce optimum separation in a minimum amount of time. The alkane hydrocarbon standard was used for the calibration of the GC before analysis of the petroleum hydrocarbon extracts.



		Sample Name	Sample Type	Cal. level	Inj.	Injection Notes	AutoLink	Tray	Vial	Injectiom Volume	Amount Std (IS, N% only)	Unid Peak Factor	Multipl
1	*	DRO	Analysis		2	none	none	Tray1	1	1.00	1	0	1
2	*	A6.1	Analysis		3	none	none	Tray1	2	1.00	1	0	1
3	*	A6.2	Analysis		3	none	none	Tray1	3	1.00	1	0	1
4	*	A6.3 MIX	Analysis		3	none	none	Tray1	4	1.00	1	0	1
5	*	A7.1	Analysis		3	none	none	Tray1	5	1.00	1	0	1
6	*	A7.2	Analysis		3	none	none	Tray1	6	1.00	1	0	1
7	*	A7.3	Analysis		3	none	none	Tray1	7	1.00	1	0	1
8	*	A8.1	Analysis		3	none	none	Tray1	8	1.00	1	0	1
9	*	A8.2	Analysis		3	none	none	Tray1	9	1.00	1	0	1
10	*	A8.3	Analysis		3	none	none	Tray1	10	1.00	1	0	1
11	*	DCM	Analysis		1	none	none	Tray1	22	1.00	1	0	1
12	*	C15	Analysis		2	none	none	Tray1	11	1.00	1	0	1
13	*	DRO	Analysis		1	none	none	Tray1	1	1.00	1	0	1
14	*	A9	Analysis		3	none	none	Tray1	12	1.00	1	0	1
15	*	A10	Analysis		3	none	none	Tray1	13	1.00	1	0	1
16	*	A11.1	Analysis		3	none	none	Tray1	14	1.00	1	0	1
17	*	A11.2	Analysis		3	none	none	Tray1	15	1.00	1	0	1
18	*	A11.3 MIX	Analysis		3	none	none	Tray1	16	1.00	1	0	1
19	*	A12.1	Analysis		3	none	none	Tray1	17	1.00	1	0	1
20	*	A12.2	Analysis		3	none	none	Tray1	18	1.00	1	0	1
21	*	AX1 coloured	Analysis		2	none	none	Tray1	19	1.00	1	0	1
22	*	AX2	Analysis		2	none	none	Tray1	20	1.00	1	0	1
23	*	AX3	Analysis		2	none	none	Tray1	21	1.00	1	0	1
24	*	DRO	Analysis		2	none	none	Tray1	1	1.00	1	0	1
25	*	DCM	Analysis		1	none	none	Tray1	22	1.00	1	0	1
26													
27													
28													

Figure 4.11 An example sample list showing sequential analysis of reference standards and sample extracts.

The steps taken to carry out the analysis of the standards and sample extracts are as follows:

- Aliquots of the standards and sample extracts were put into well labelled 2.2 mL GC vials and placed in the sample tray 1 and 1 µl of the sample

injected by the Combi PAL auto sampler in accordance with the sample list.

- The reference standards were run first followed by the sample and the blanks and so on. Each analysis was carried out in triplicate and the numerical order of the sample list was done automatically.

- DCM blanks were run intermediately at random to flush out the injection port and column to check that the column was not retaining any residual matter (refer to Figure 4.11)

- Alkane hydrocarbon standard mixtures were analysed after every ten samples. This served to check that the injections were consistent with minimal drift in the calibration. This was done to ensure that the instrument performed at its optimum and served as a calibration step for the instrument. Figure 4.12 were the overlaid chromatograms of the alkane reference standard mixtures (DRO, C10-C25 & Alkane mix C10-C34).

- BTEX samples were run along with the alkane reference standards and sample to calibrate the instrument for BTEX range organics.

- Prepared laboratory standards were also run to compare and confirm peaks generated from the alkane standard mixtures the with samples.

- All samples were analysed several times to assess the reproducibility of the chromatographic method. A statistical analysis was performed on the sample to determine whether any significant level of variation existed during the analysis of hydrocarbons by the method optimized (refer to Appendix III A).

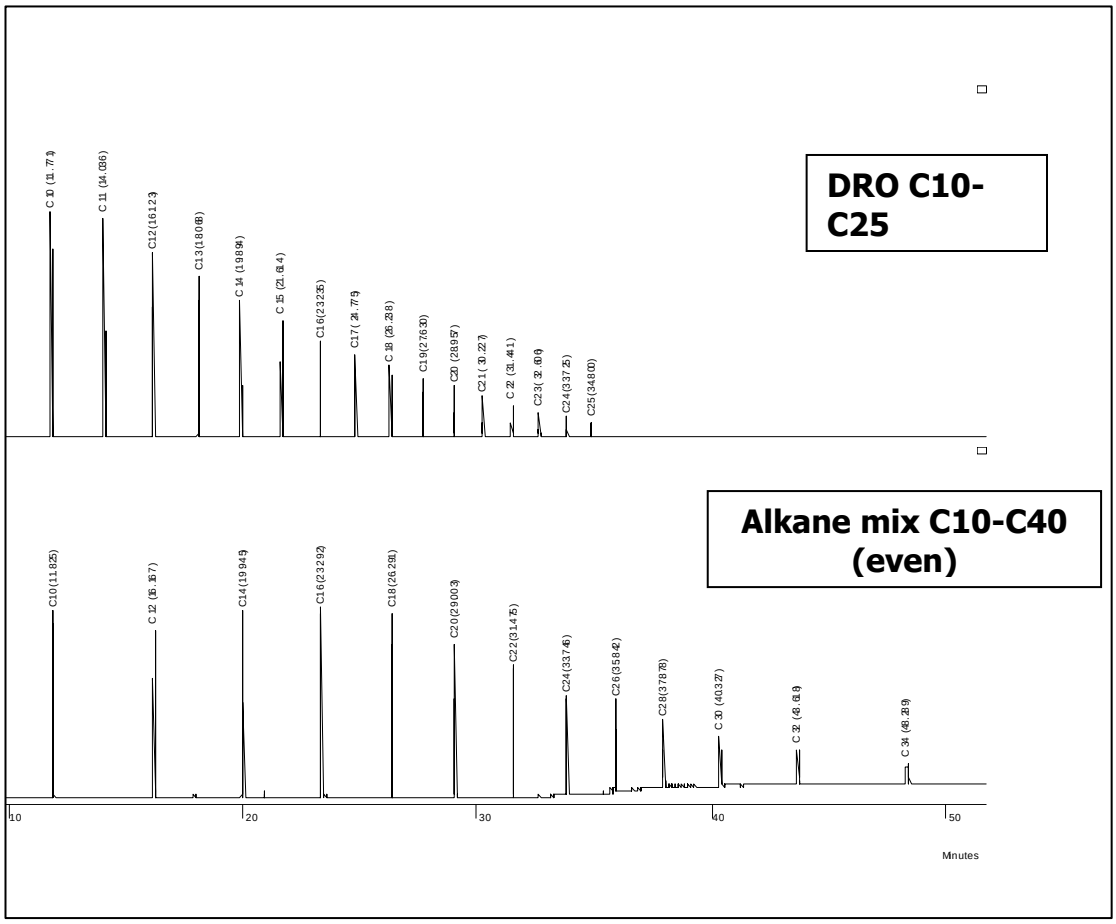


Figure 4.12 **Chromatograms of the reference standards. Above: DRO mix (16 hydrocarbons, C10-C25) Below: Alkane mix (16 separated Hydrocarbons, C10 –C34), even number of carbons only**

The peaks and retention times established by the two standards in figure 4.12 were compared with each other and confirmed by running the standards prepared in the laboratory such C10, C11, C14, C15 and the tcd. Their retention times conformed to that of the reference standards. Figure 4.13 showed the chromatograms of prepared lab standards.

Tentative identification of analyte occurs when a peak from the sample extract falls within the daily retention time window (RTW). Confirmation is made by further analysis using the other reference standards and the ones prepared from the laboratory as shown in figures 4.13-4.15.

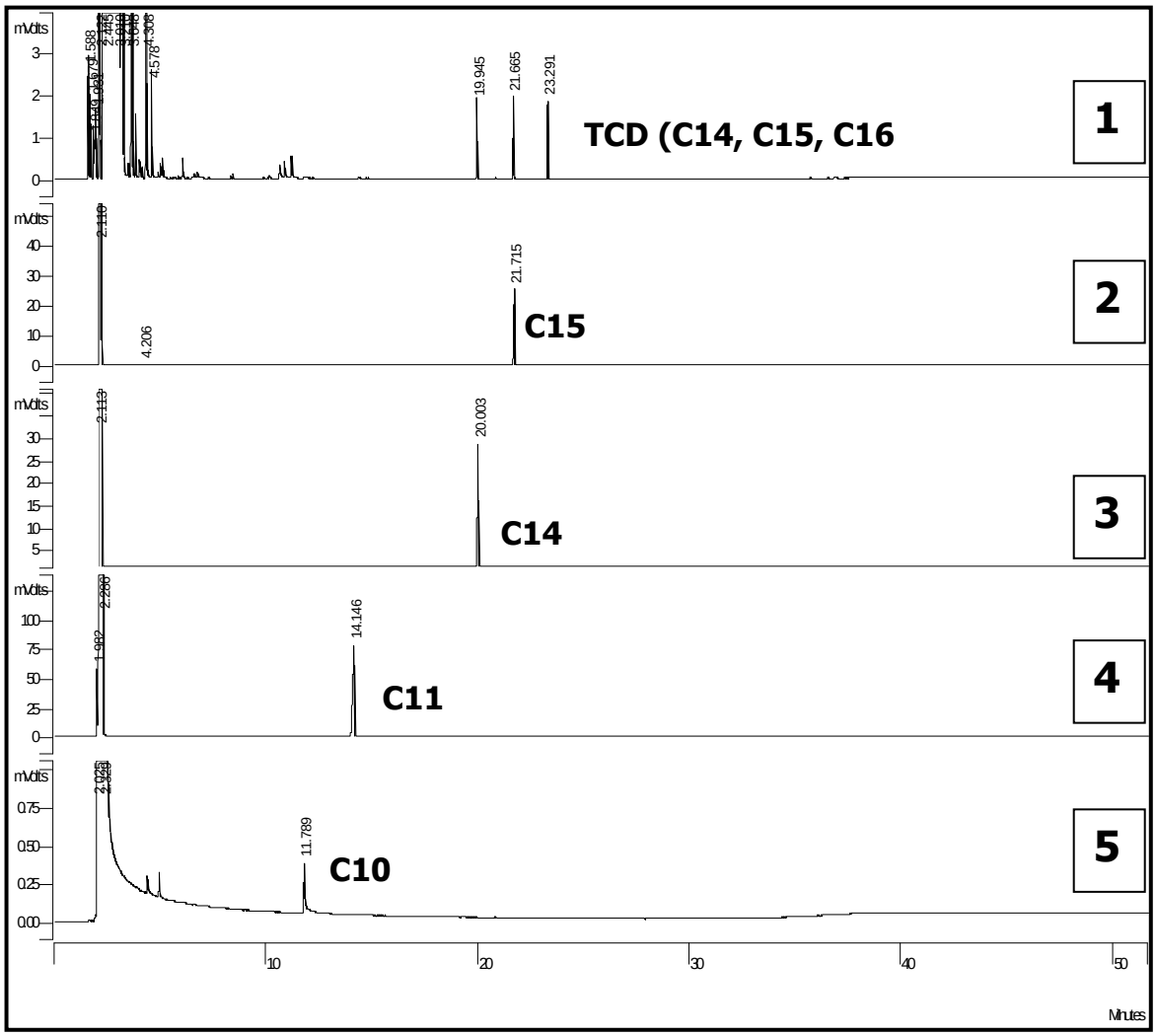


Figure 4.13 **Chromatograms of standards prepared in the laboratory. 1) = TCD (thermal conductivity detector mix), 2) = Pentadecane ($C_{15}H_{32}$), 3) = Tetradecane ($C_{14}H_{30}$), 4) =), Undecane ($C_{11}H_{24}$), 5) = Decane ($C_{10}H_{22}$)**

Chromatograms of Figure 4.13 were compared with that of alkane reference standard mix to establish and confirm the peaks obtained from it.

Figure 4.14 showed how all the laboratory standards prepared were used to confirm the peaks in the reference Alkane standard mix.

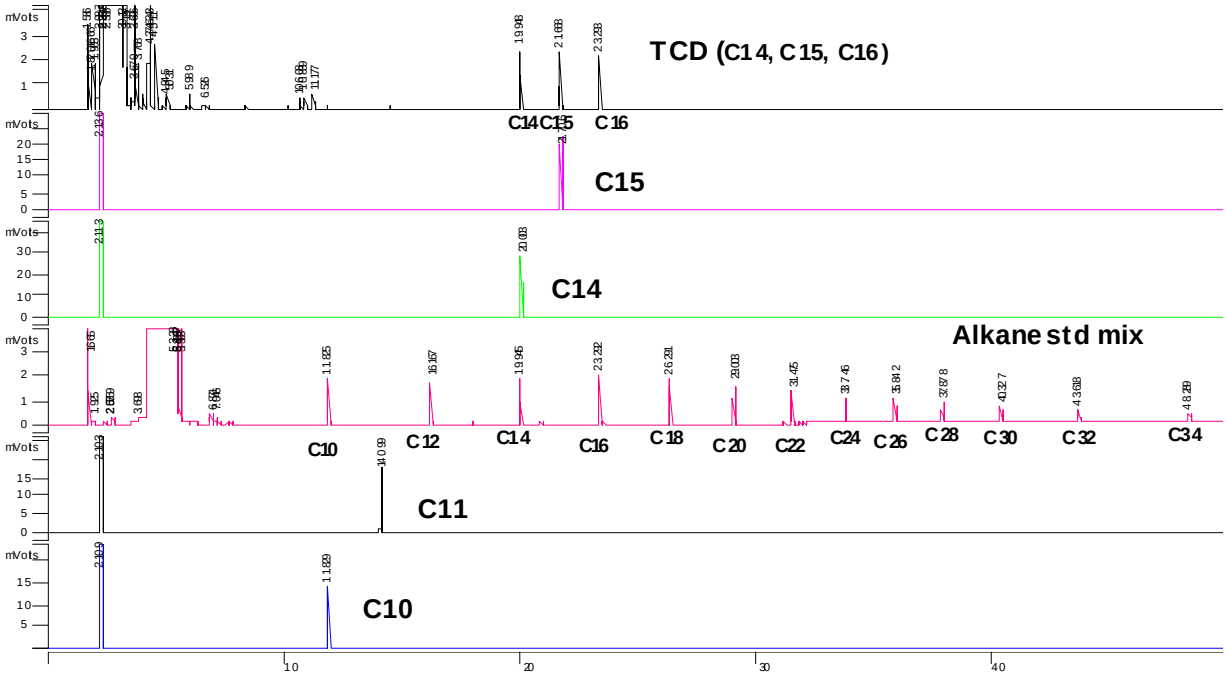


Figure 4.14 **Overlaid chromatograms of the alkane standard mix and laboratory standards used to confirm the authenticity of the calibration procedures by matching the retention times**

Window retention times are crucial to the identification of target compounds. Standard solution (C10 & C40) for the determination of the retention time widow (RTW) was injected to calibrate and fix RTW from C10 and C40 before the standards and sample measurements. Therefore, RTWs were established to compensate for the minor shifts in absolute retention times as a result of sample loadings and normal chromatographic variability. However, figure 4.15 showed the chromatograms of tcd having significant peaks at C14, C15 & C16 compared with the two references. RTW was established as part of the method validation.

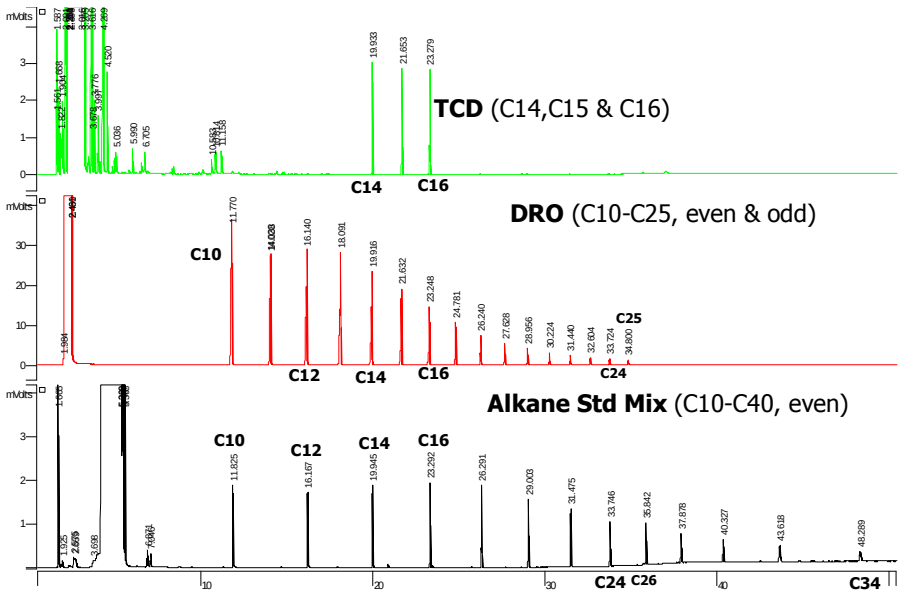


Figure 4.15 **Retention times of Alkane standard mix, DRO and TCD were established after the RTW validation**

4.6 Evaluation

The method for the analysis of petroleum hydrocarbon has been optimally developed, tested and the RTW established for all the standards and samples. Necessary operational checks were carried out to ensure that the calibration of the instrument and sample was reproducible.

The following evaluation was necessary in order to meet the objectives of this work:

1. The total petroleum hydrocarbon (TPH) content (Chapter 5, table 5.6) was determined at each sampling points in the sampling area. The amount of hydrocarbons found at the three levels of each point was also compared.

2. Penetration and spatial distribution of a range of hydrocarbons (C10-C26 and C26 –C34) within the soil levels was assessed (refer chapter 5, figures 5.13-5.24).
3. Percentage distribution and classification of a range of hydrocarbons in the soil depth was considered.
4. Chemical similarities and differences among the contaminant compounds in the various sampling spots were established by Chemometric cluster analysis method (chapter 5, figures 5.26-5.27).

The hydrocarbon content (C) for each sample was computed according to the following equation [18]:

$$Cs = \frac{Cc \,.\, Vt \,.\, D}{Ws} \; X \; \frac{1\ mg}{1000\ \mu g}$$

Where:

Cs = Concentration of total hydrocarbon petroleum (THP) in $mgkg^{-1}$ or mgL^{-1} in the samples.

Cc = Concentration from calibration curve in μgmL^{-1}. (If CF is used for calculations, then this value is the area calibration/CF)

CF = Calibration factor for the standard

$$= \frac{\text{Total area of calibration standard (average of six readings)}}{\text{Concentration of calibration standard } (mgL^{-1})}$$

Vt = Volume of extract (mL)

D = Dilution factor if sample or extract was diluted prior to analysis. If no dilution was made, D= 1, dimensionless.

Ws = Weight of sample extracted (in grams). If units of Kg was used the result is multiplied by1000 g/kg. If water sample, then the unit is L.

From the above application, the CF value was calculated as follows:

The total area of calibration was determined by taking the average of the areas analyzed for the standards (peak areas) and the concentration of the alkane standard mix used was 50 mgL^{-1}.
Average peak area of calibration standard was computed to be 2857.72 from results of analysis and that of all the samples.

Then CF = 2857.72/50 = 57.15

The concentration for TPH, Cs was calculated for all the samples in the three levels of top soil (0-15 cm), middle (15-30 cm) and 30-60 cm for the last level analyzed (see chapter 5, table 5.6).
An example of such calculation is shown below for sample A1 for all the three levels as A1.1 (0-15 cm), top soil, A1.2 (15-30 cm), middle level and A1.3 at level 30-60 cm. Similarly all other samples are coded in like manner.
For example, A1.1, weight of sample = 10.1672 x 10^{-3} kg,
Volume of extract used = 100 mL

Cs = concentration of hydrocarbons, TPH in soil A1.1 (top soil)
= (548.57/50) µg/mL X 100 mL X 1 mg/1000 µg /10.1672 x 10^{-3} kg
= 93.89 $mgkg^{-1}$
≈ 94 $mgkg^{-1}$ for sample A1.1 (top soil)

Sample A1.2, middle soil level, 15-30 cm.
Weight of sample = 10.0099 x 10^{-3} kg, volume of extract used = 100 mL.

Cs = 155.47 $mgkg^{-1}$

= 256 $mgkg^{-1}$

Similarly, the Cs was computed for sample A1 for the last level A1.3 (30-60 cm).
Weight of sample = 10.0621 x 10^{-3} kg, volume of extract used = 100 mL.

Cs = 75 $mgkg^{-1}$

Table 5.6 showed the computed concentration of hydrocarbons in all the samples including control samples.

The results and discussion are presented in chapter 5.

4.7 Conclusion

The sampling method was established and the sample preparation and optimized extraction were achieved. The GC-FID analysis of the petroleum contaminated soil extracts using the optimized methods yielded higher concentration of petroleum hydrocarbons than the control soil samples.

Hydrocarbon reference standards were used to assess the level of contaminated in the soils of the study site.

The smallest concentration of hydrocarbon was recorded in the deepest level of soil measured while greatest concentration occurred in the middle soil level.

4.8 References

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CHAPTER 5

RESULTS AND DISCUSSION

5.1 Data Analysis

All data generated from the GC instrument including details of the instrument parameters, were recorded into the proprietary Varian Star™ software that automatically saved the experimental information into the Microsoft Excel File format. Microsoft Excel software was subsequently used to process the data shown in this chapter. The information needed from the experimental data was, sample identity, retention time and sample concentration (expressed as the peak area counts). Replicate analysis was necessary to establish reproducibility and ensure good precision of the analysis.

5.2 Multivariate Analysis

The chemical information generated from this work was inherently multivariate meaning that more than one measurement or variable were made on a single sample. Statistical evaluation of these soils was done to determine the chemical similarity of the petroleum hydrocarbon contaminants by pattern recognition using cluster analysis (refer to section 5.8). Cluster analysis of observations is a powerful and useful tool in establishing the existence of closely related classes. Cluster analysis of petroleum hydrocarbons was carried out by considering correlated variables ranging from the concentration of the samples to identity of the samples.

A dendrogram with cluster observations and cluster variables were produced (figure 5.28) to confirm the similarity groupings of the sample.

5. 3 Sampling

Table 5.1 showed the sample identity, number of samples and depths of sampling from the site locations.

Table 5.1 Total Number of Samples and Depths of sampling.

Location	Sample identity	No. of samples	Sample depth (cm)
SITE 1A	A1 to A8	23	0-15 15-30 30-60
SITE 1B	A9 to A12	12	0-15 15-30 30-60
Site C	AX12, AX13 AX1, AX2	12	0-15 15-30 30-60
	Total =	**47**	

Site investigation survey was carried out according to [1].
Sampling distances were taken from the Well head as a reference point.

5.4 Extraction

DCM proved to be the most suitable solvent over hexane, acetone and toluene for this extraction due to its consistency, efficiency and ability of not interfering with BTEX RTW between C5-C9.

5.4.1 Confirmation of Optimum Extraction Time

The parameter that was needed to be established was the optimum solvent extraction time for the soil samples. The optimum extraction time that was used for this work was established using DCM as the solvent choice. Table 5.2 shows

the mass used for each hourly extract, the residue obtained after evaporation of the extract and the corresponding percentage yield.

Table 5.2 Average mass yields produced for the hourly extractions of a soil sample A3 using toluene.

Extraction Time (Hour)	Sample mass (g)	Sample Residue (g)	Yield (%)
1	10.0624	0.2479	2.46
2	10.0196	0.2921	2.91
3	10.0863	0.3116	3.08
4	10.1008	0.3261	3.22
5	10.0021	0.3229	3.22

$$\text{Percentage (\%) Yield} = \frac{\text{Mass of Extract}}{\text{Mass of sample}} \times 100$$

The average percentage recovery was 73.7% ± 26.

$$\%\ R = \left[\frac{Co}{Cs} \right] \times 100$$

Cs = average concentration of sample ≅ peak area of sample

Co = Concentration of Internal Standard ≅ peak area of Int. standard

Figure 5.1 illustrates the plot of the time of extraction (x-axis) against percentage residue yield (y-axis).

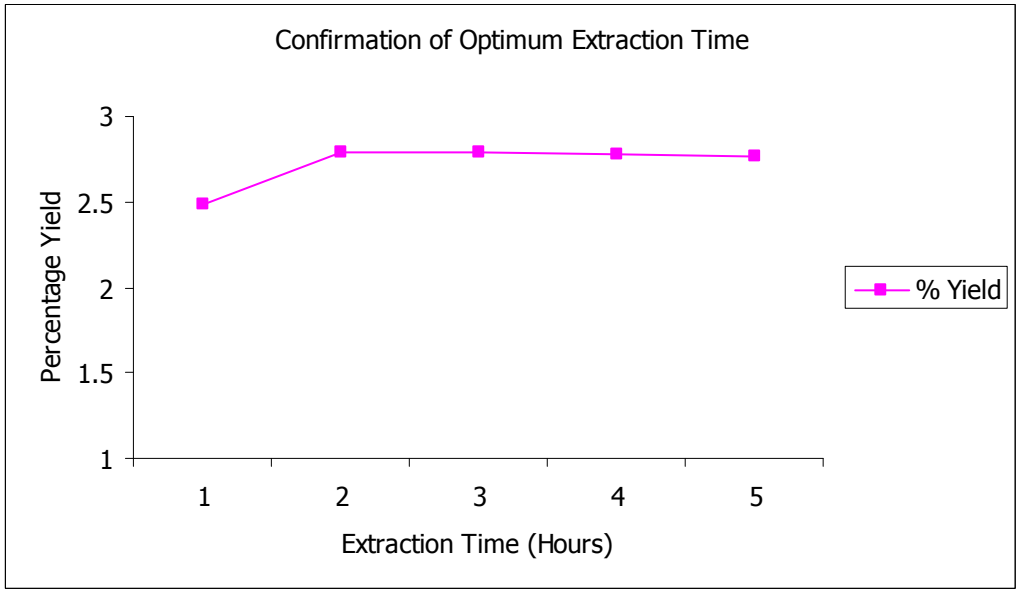


Figure 5.1 Plot of average percentage yield of extract obtained with time of extraction using DCM as solvent.

Figure 5.1 shows that extraction time of two hours at optimum and beyond which further extractions reached a yield plateau, hence the selection of two hours as the time for extraction. The two-hour extraction time chosen was the logical choice for the completion of extractions within a reasonable time.

The choice of extraction method amidst other recent extraction techniques include its peculiar application, availability in the laboratory and the ease of setting up with minimal or no cost among other advantages outlined in chapter 4.3.1. The drawbacks of the extraction method include higher solvent consumption, long time and possible contamination.

Recent automatic devices have been developed to ease extraction. Contrary to Soxhlet extraction, SFE and MAE do not require large volumes of solvents and may be rapid techniques for extraction of organic compounds. However, compared with Soxhlet extraction apparatus, SFE is fairly expensive, difficult to use and makes use of limited amount of samples, about 0.5- 5 g. Good repeatability could be obtained by at least 5 g sample which will otherwise create adequate sample representation.

Previous studies carried out by [5] and [6] have revealed that SFE-IR and GC-IR methods have produced results that were comparable to those obtained by

using a standard Soxhlet extraction. Report [7] portrayed that amount of samples achieved with Soxhlet extraction is consistent.

All samples were cleaned up by passing them through Florisil® and anhydrous sodium sulphate SPE tube to remove moisture, impurities and substances that could interfere with the final determination and quantitation of target analytes was vividly explained in Chapter 4.3.4.

5.5 Chromatography

A readily accessible, reliably fast and economically favourable GC-FID method was available for the qualitative and quantitative determination of Petroleum Hydrocarbons in different soil layers. A 30 m capillary column was employed for the separation. RTW, BTEX (GRO), Alkane Mix- WOO (C10-C40), DRO (C10-C25 for16 compounds) were used as reference standards along side laboratory prepared standards to calibrate and validate the instrument (refer to Chapter 4.4.3 -4.4.4).

DCM was the suitable solvent used; its elution peak did not confuse with carbon numbers C5-C9 (BTEX) during the analysis like any other hydrocarbon or PAH would have done.

5.5.1 Column Conditioning and Operational Checks

In order to ensure proper functioning of the instrument, reproducibility and repeatability of the analysis, column conditioning and routine operational checks were carried out. The manufacturer's test mixture was run on the optimized GC-FID method developed in chapter 4.4.5 and the chromatogram compared to be similar to the supplier's. A test mix provided was analysed to establish that the parameters and operating conditions met the manufacturer's specifications. Several blank runs followed by retention time window standard (C10 –C40) were carried out to ensure reproducibility and repeatability [8]. Operational checks continued as blank injections were performed making the column free from any contaminant. Any trace amounts of impurity evident from the blank analysis were flushed out by several injections of DCM until there was no

evidence of contamination of the column. Blanks extracts and fine sand extracts were also run to ensure that the extraction system did not introduce hydrocarbon or other contamination. All the liquid injection volumes were 1 µl and performed by Combi PAL auto sampler. Figures 5.2 & 5.3 show typical chromatograms of solvent (DCM) blank, extracts of silver sand and blank thimble extraction.

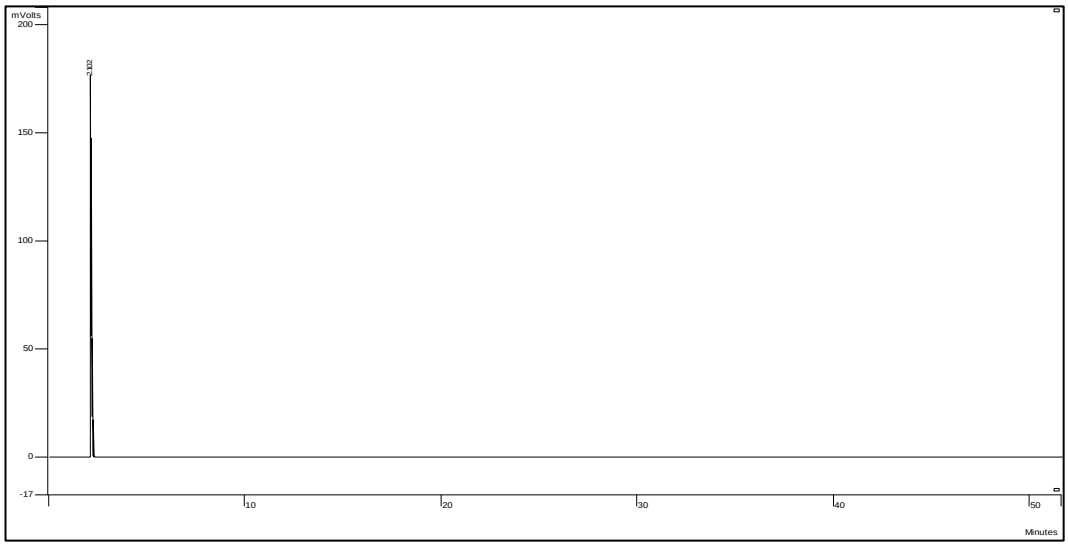


Figure 5.2 Chromatogram of DCM

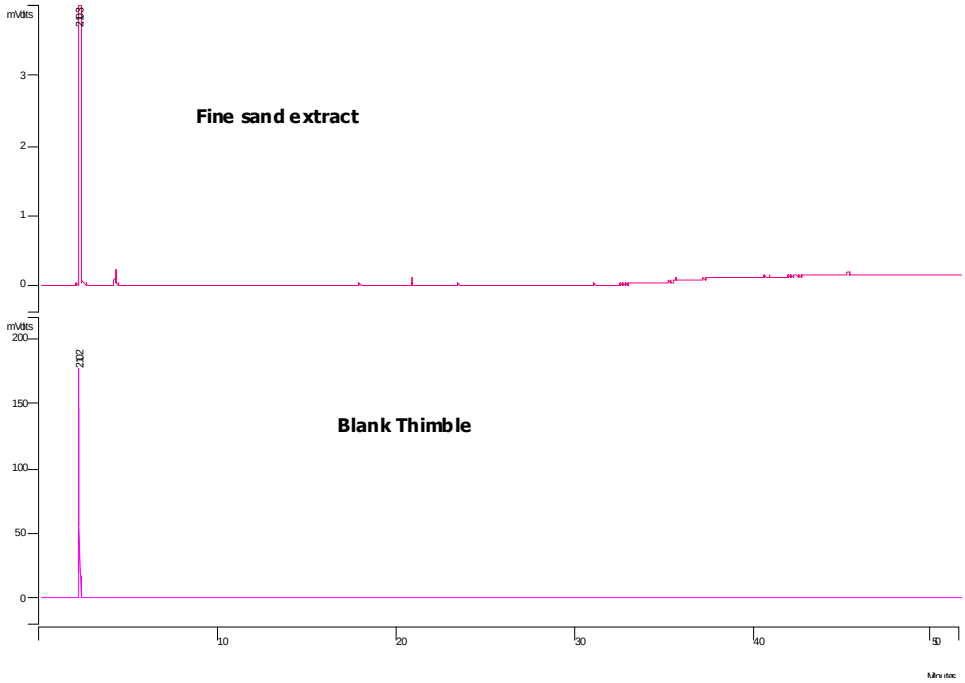


Figure 5.3 (Above) Chromatogram of extracts of silver sand
(Below) Chromatogram of thimble blank extract
Both peaks showed no significant contamination.

Figure 5.2 is the chromatogram of dichloromethane (DCM) used as a solvent. The chromatogram showed that the column is clean and no carry over of previous injections or any contaminants. Figure 5.3 shows the extract of the silver sand (no petroleum spill) and the thimble extract without the soil sample. The chromatograms were neat, linear with no indication of possible contamination in the column. These proved that the extraction apparatus was not contaminated during the process.

5.5.2 Hydrocarbon standards and Retention Times

Procured hydrocarbon standards were run in triplicates and their retention times compared with 1000 ppm of each of decane (C10), undecane (C11), tetradecane (C14), pentadecane and characteristic thermal conductivity mix at peaks C14, C15 & C16. The hydrocarbon standards were (i) Alkane Mix, C10-C40 (even carbon number), (ii) DRO, C10-C25 (iii) BTEX (benzene, toluene, ethylbenzene and total xylene) (iv) Retention Time window RTW, C10 & C40.
Table 5.3 below shows the retention times obtained for the hydrocarbon standard used during analysis. The average values shown were used for the calibration of the instrument and analysis of the extracts. The standard deviations, % RSD at 95 % confidence level obtained was shown on the table.

Table 5.3 Chromatographic information of Alkane Standard Mix, C10-C40 (even) showing separation up to C34.

Standard Mixture for n- Alkanes even C10-C40

Name	Ret Time	PEAK AREAS (Counts)						
	(min)	1	2	3	AV. PK. Area			
					MEAN	STD DEV	% RSD	95% CL
n-Decane (C_4H_{22})	11.82	3750	3751	3752	3751.0	1.00	0.03	3.04
n-Dodecane C_4H4	16.17	3498	3501	3499	3499.3	1.53	0.04	4.65
n-Tetradecane, $C_{14}H_{30}$	19.95	3876	3879	3878	3877.7	1.53	0.04	4.65
n-Hexadecane, $C_{16}H_{36}$	23.29	4236	4239	4237	4237.3	1.53	0.04	4.65
n-Octadecane $C_{18}H_{38}$	26.29	4048	4051	4047	4048.7	2.08	0.05	6.33
n-Eicosane, $C_{20}H_{42}$	29	3463	3465	3466	3464.7	1.53	0.04	4.65
n-Docosane, $C_{22}H_{46}$	31.48	2987	2990	2989	2988.7	1.53	0.05	4.65
n-Tetracosane, $C_{24}H_{50}$	33.75	2265	2267	2268	2266.7	1.53	0.07	4.65
n-Hexacosane, $C_{26}H_{54}$	35.84	2247	2251	2249	2249.0	2.00	0.09	6.09
n-Octacosane, $C_{28}H_{58}$	37.88	1792	1794	1795	1793.7	1.53	0.09	4.65
n-Triacontane, C_{30} H_{62}	40.33	1794	1792	1790	1792.0	2.00	0.11	6.09
n-Dotriacontane, $C_{32}H_{66}$	43.62	1688	1690	1689	1689.0	1.00	0.06	3.04
n-Tetratriacontane, $C_{34}H_{70}$	48.29	1494	1491	1493	1492.7	1.53	0.10	4.65

NB: Calculation of standard deviation on 3 replicates calculated automatically from the instruments software.

Ret. Time = Retention time

STD DEV = standard deviation

RSD = Relative standard deviation

CL = Confidence level

AV. PK = Average peak

Table 5.4 Chromatographic information of DRO reference standard mix, C10-C25 (16 compounds) fully separated.

DRO Mix (16 Components)								
DRO Mix	Ret Time	Mean Av. Peak counts	(3 REPLICATES PEAK. AREA)			St Dev	% RSD	95% CL
C10 Decane	11.76	49875.0	49873	49877	49875	2	0.00	6.09
C11 Undecane	14.02	46776.7	46776	46781	46773	4.04	0.01	12.3
C12 Dodecane	16.11	39223.0	39221	39225	39223	2.00	0.01	6.09
C13 Tridecane	18.06	33386.0	33382	33390	33386	4.00	0.01	12.2
C14 Tetradecane	19.87	28682.3	28684	28681	28682	1.53	0.01	4.65
C15 Pentadecane	21.61	24687.0	24688	24689	24684	2.65	0.01	8.05
C16 Hexadecane	23.23	20679.0	20676	20682	20679	3.00	0.01	9.13
C17 Heptadecane	24.77	17668.0	17668	17670	17666	2.00	0.01	6.09
C18 Octadecane	26.23	15425.0	15422	15429	15424	3.61	0.02	10.9
C19 Nonadecane	27.62	12557.0	12553	12561	12557	4.00	0.03	12.17
C20 Eicosane	28.95	10376.7	10377	10374	10379	2.52	0.02	7.66
C21 Heneicosane	30.22	8341.00	8338	8345	8340	3.61	0.04	10.97
C22 Docosane	31.44	6581.67	6585	6579	6581	3.06	0.05	9.30
C23 Tricosane	32.6	5108.00	5106	5110	5108	2.00	0.04	6.09
C24 Tetracosane	33.72	3887.67	3883	3892	3888	4.51	0.12	13.72
C25 Pentacosane	34.8	2885.00	2883	2887	2885	2	0.07	6.09

NB: Calculation of standard deviation on 3 replicates was calculated automatically from the instruments software.

Figures 5.4 illustrates the instrument chromatograms and figure 5.5 is obtained from the Microsoft Excel using the analyzed data information from tables 5.3 & 5.4 for Alkane standard mix and DRO standard mix.

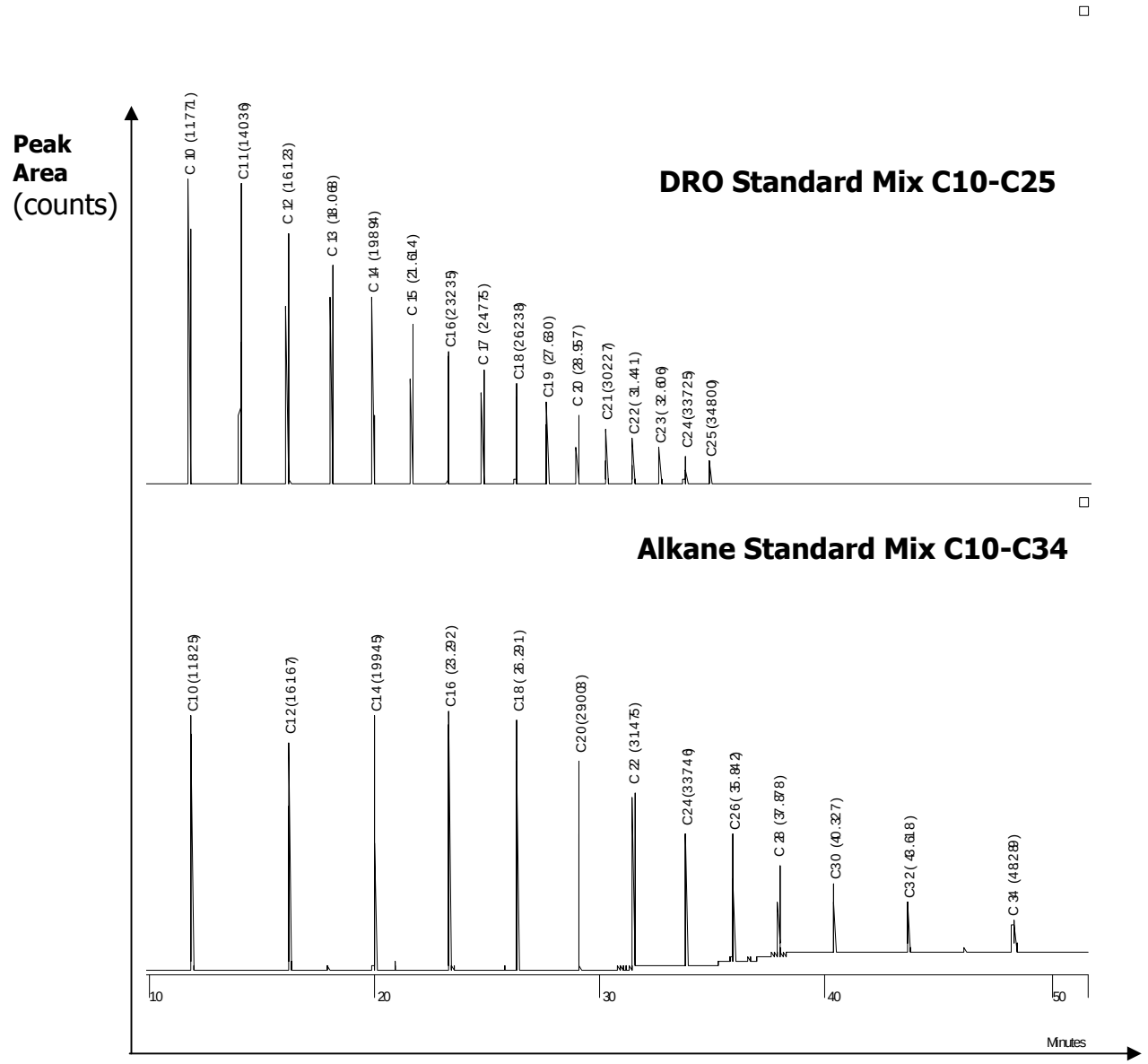
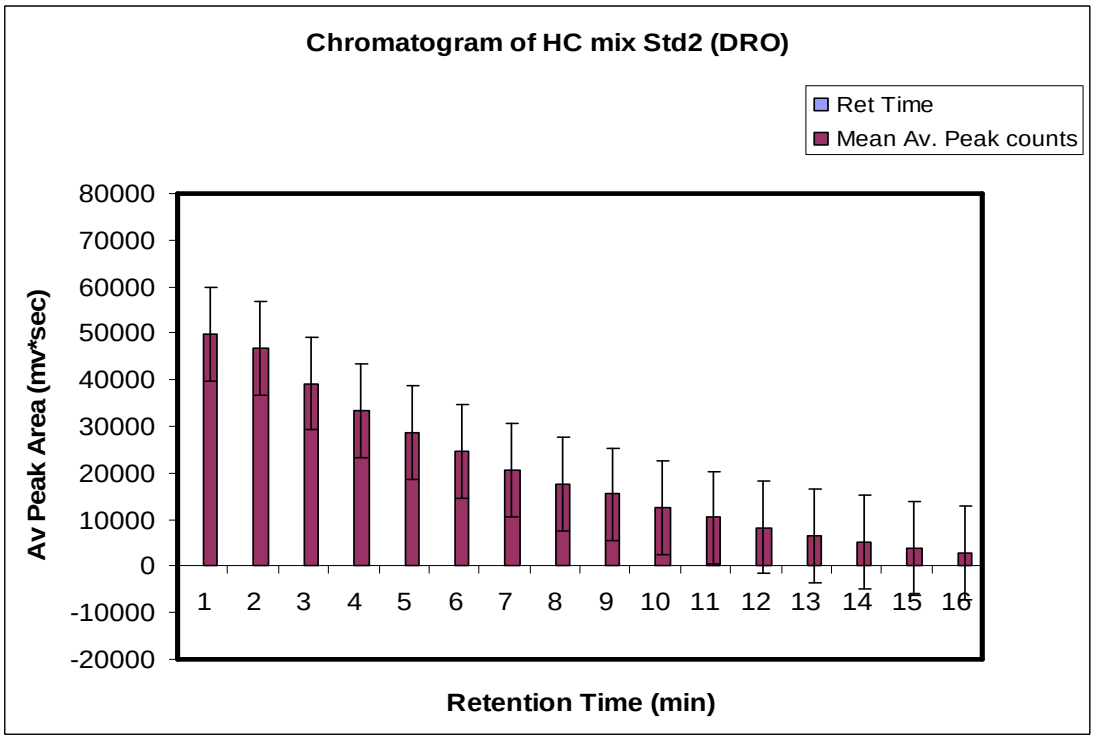


Figure 5.4 Comparing GC-FID's chromatograms of Alkane and DRO standard mixtures. Numbers in bracket indicate the retention times.



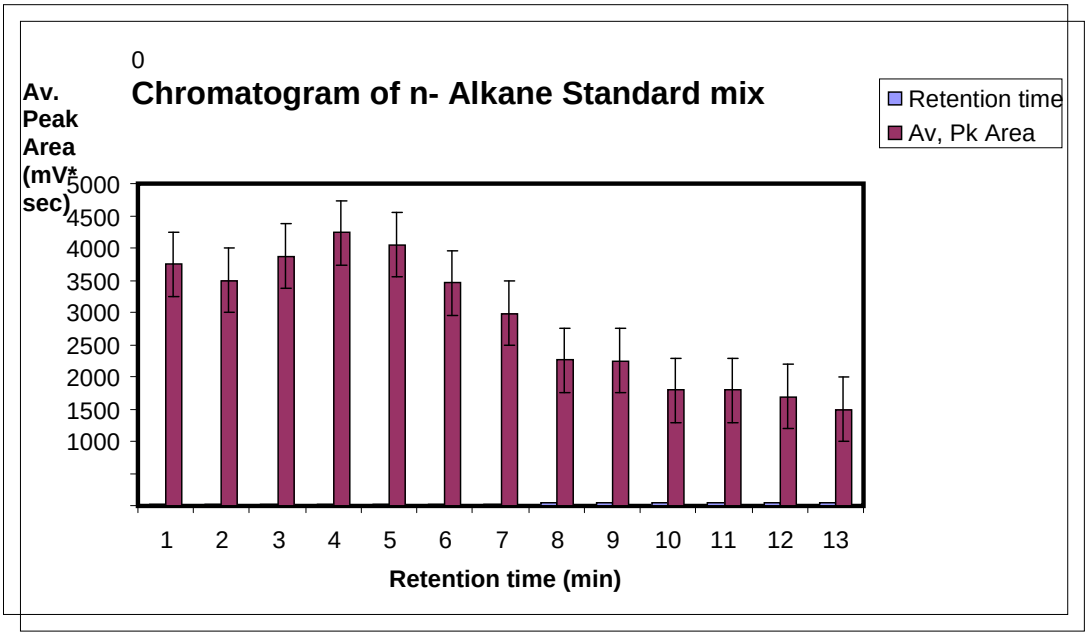


Figure 5.5 Concentrations and retention times of DRO (above) and Alkane (below) standard mixtures using Star™ software data on Microsoft Excel to show the repeatability of the analysis.

Two specific reference standards were used in calibrating, identifying and validating the compounds in the analyzed samples. Alkane standard mix had even numbers of hydrocarbons from C10 to C40 and instrument separated up to C34. The odd number of hydrocarbons were undertaken by the simultaneous analysis of DRO standard mix up to C25. Odd number peaks in the samples that occurred at C27, C29, C31 and C33 which were not covered by both standards were identified by Kovats Indices using the formula below using the retention time information in table 5.5.

$$I^T = 100 \left(\frac{t^T_{Ri} - t^T_{Rz}}{t^T_{R\,(Z+1)} - t^T_{Rz}} + Z \right) \quad \text{..... } \mathbf{5.1}$$

Where:

I^T = retention index for temperature programmed GC analysis

t^T_{Ri} = retention time of sample peak

t^T_{Rz} = retention time of n-alkane peak eluting immediately before sample peak

$t^T_{R\,(Z+1)}$ =retention time of n-alkane peak eluting immediately after sample peak

z = carbon number of n-alkane peak eluting before sample peak

Table 5.5 showed the retention times of the two calibrated standards. The values recorded in black and red colours refer to retention times for Alkane Mix and DRO mix respectively. Retention times for C27, C29, C31 and C33 that were not covered by the standards were verified by Kovats retention time index formula 1 above. Hydrocarbons present in the samples were validated by the retention times of the certified reference standards.

The retention times recorded from the analyzed samples fell between 21.00 and 48.28 minutes as indicated in table below.

Table 5.5 Compared Retention Times of the Chromatographic Analysis of Hydrocarbon Reference Standards 1 (Alkane mix) and 2 (DRO mix).

Carbon Number	Hydrocarbon Standard mix 1 (C10-C40)	Hydrocarbon Standard mix 2, DRO (C10-C25	Retention Time (min)
C10	Decane	Decane	11.8
C11	-	Undecane	14.02
C12	Dodecane	Dodecane	16.1
C13	-	Tridecane	18.1
C14	Tetradecane	Tetradecane	19.9
C15	-	Pentadecane	21.6
C16	Hexadecane	Hexadecane	23.2
C17	-	Heptadecane	24.8
C18	Octadecane	Octadecane	26.2
C19	-	Nonadecane	27.6
C20	Eicosane	Eicosane	29.0
C21	-	Heneicosane	30.2
C22	Docosane	Docosane	31.4
C23	-	Tricosane	32.6
C24	Tetracosane	Tetracosane	33.7
C25	-	Pentacosane	34.8
C26	Hexacosane	-	35.8
C27	-	-	-
C28	Octacosane	-	37.9
C29	-	-	-
C30	Triacontane	-	40.3
C31	-	-	-
C32	Dotriacontane	-	43.6
C33	-	-	-
C34	Tetratriacontane	-	48.3

The table displays the retention times for the two reference standards (Alkane mix and DRO mix) with the DRO mix recorded in red. The retention times of the samples were compared with the standards to identify the compounds in the samples. The instrument did not separate Alkane mix up to C40 but stopped at $C_{34}H_{70}$, tetratriacontane. The even number hydrocarbons in both standards had the same retention times as shown on the table.

Laboratory standards (C10, C11, C14, C15, C16) were prepared, analyzed and overlaid with the reference standards to confirm their identity and retention times as shown in figures 5.6 and 5.7 as parts of the validation and operational checks.

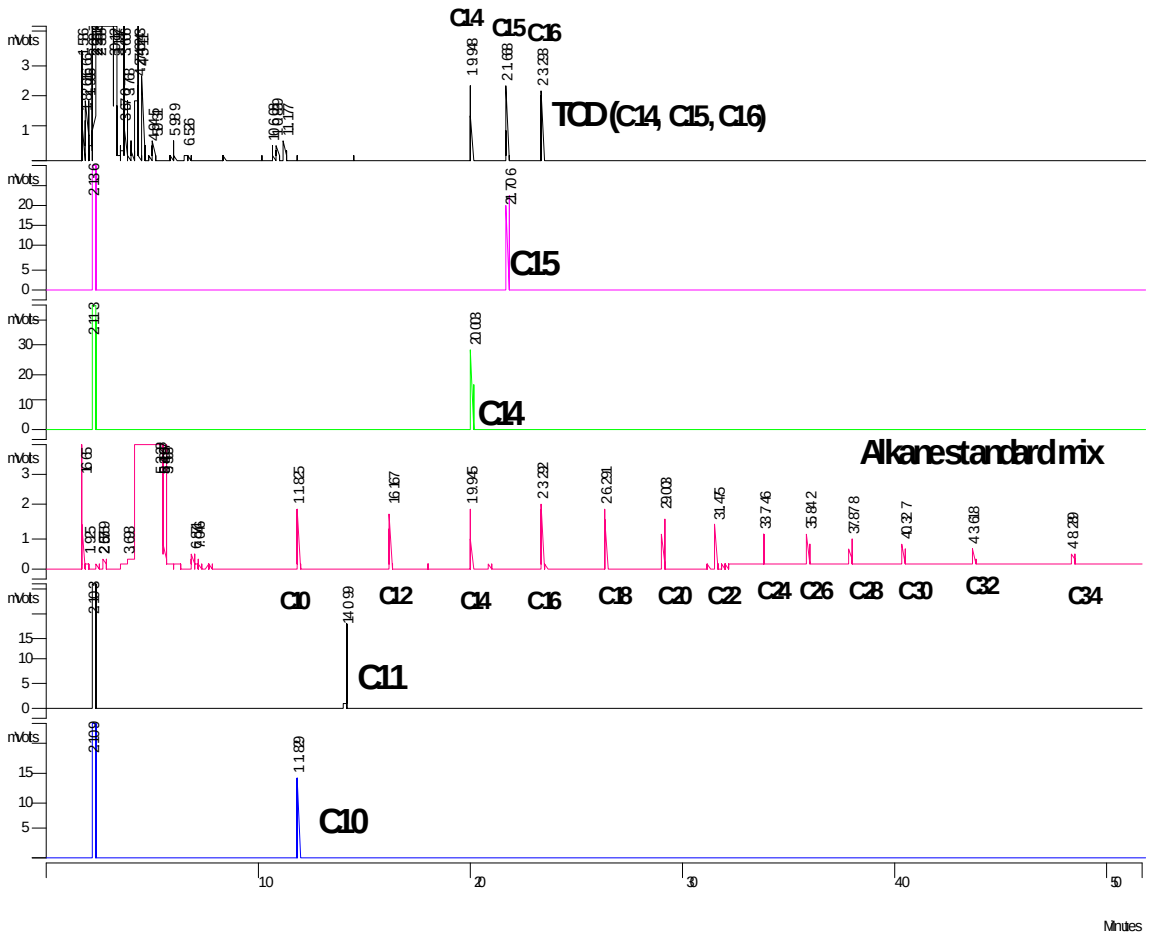


Figure 5.6 Alkane standard mix overlaid with lab standards, C1O, C11, C14, C15 and C16 to confirm the identity and retention times of the standard. Chromatogram of the Alkane mix was prepared in hexane, hence the interference in retention time at C5-C8. TCD was similarly prepared in heptane while all lab samples were prepared with DCM-the extraction solvent.

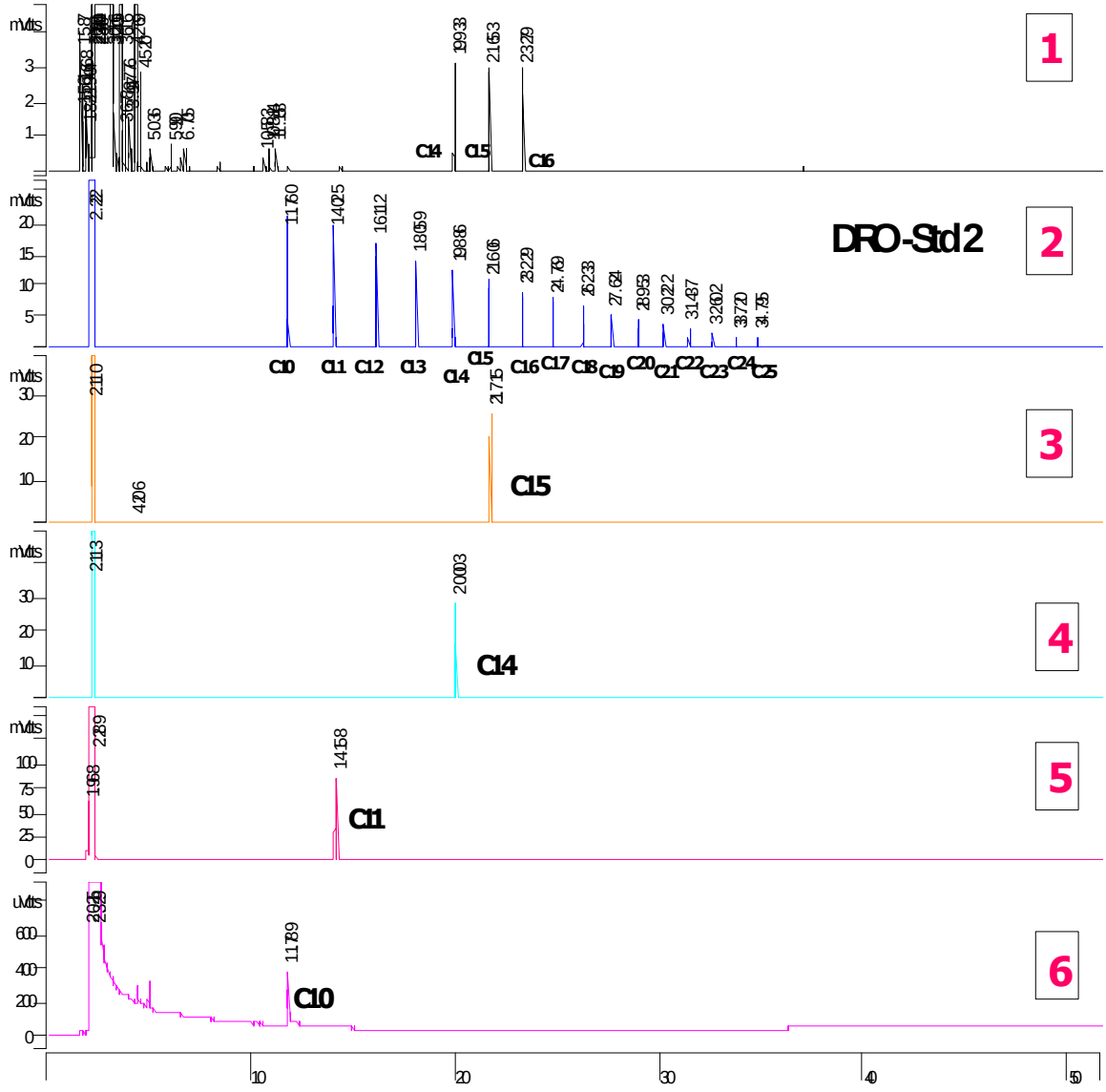


Figure 5.7 **DRO standard mix in Chromatogram No. 2 is overlaid with lab. Standards to confirm the identity and retention times of the samples. DRO and other lab standards were prepared with DCM, hence no interference at retention time window C5-C9. Chromatogram No.1 was the TCD and was the only lab standard prepared with a hydrocarbon, heptane, hence appearance of various isomeric peaks between C5-C9.**

The retention times of the reference standards were confirmed by running known concentrations of the laboratory standards. Apart from the Alkane reference standards used to determine the DRO and WOO compounds in samples, BTEX standard, prepared in methanol, was run concurrently with them to establish the retention times of the volatile gasoline organics. Figure 5.8 shows the chromatogram of BTEX standard.

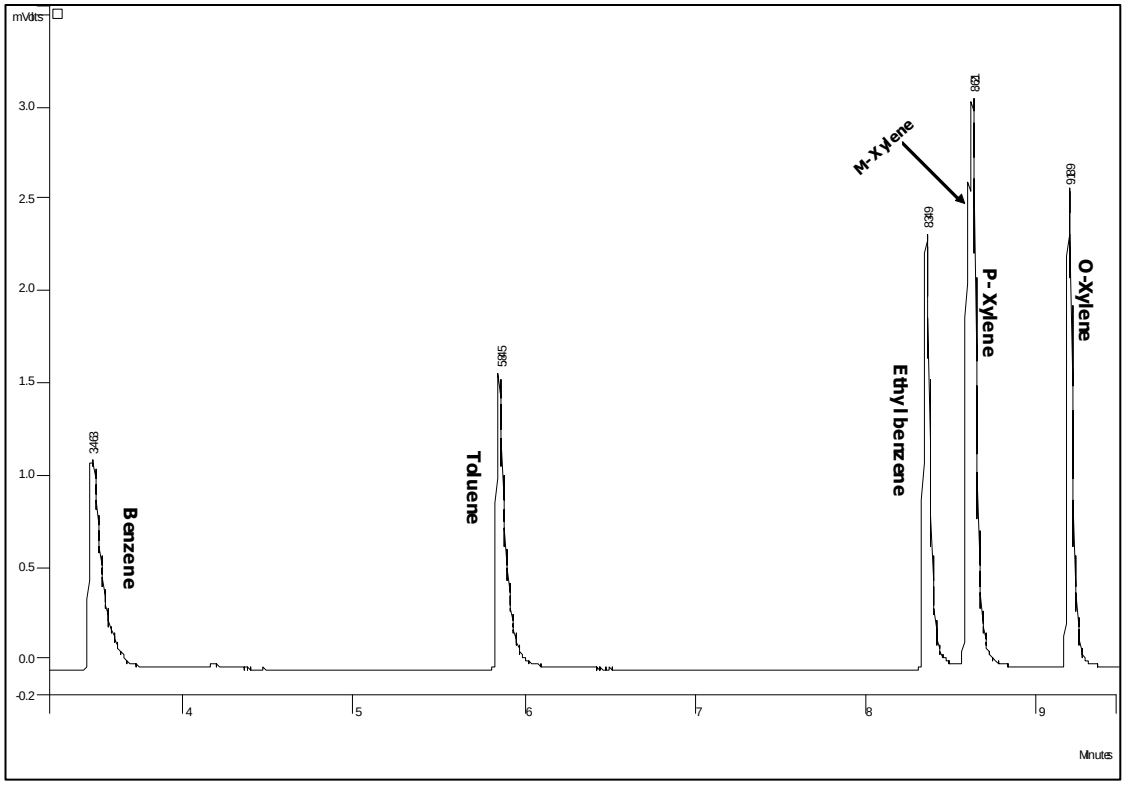


Figure 5.8 Chromatogram of 1000 ppm BTEX standard in methanol.

Benzene came out at retention time 3.4 minutes and O-xylene eluted last at 9.2 minutes thereby occupying the RTW between C5-C9 just before the retention time of the Alkane and DRO standard mixtures. This showed that peaks found in the analyzed sample within that retention time range would portray BTEX presence in the sample and the absence of such peaks would mean otherwise.

Further investigation to the authenticity of the retention times of the reference standards were carried out by comparing their chromatograms with sample 3.1 (top soil) as shown in figure 5.9.

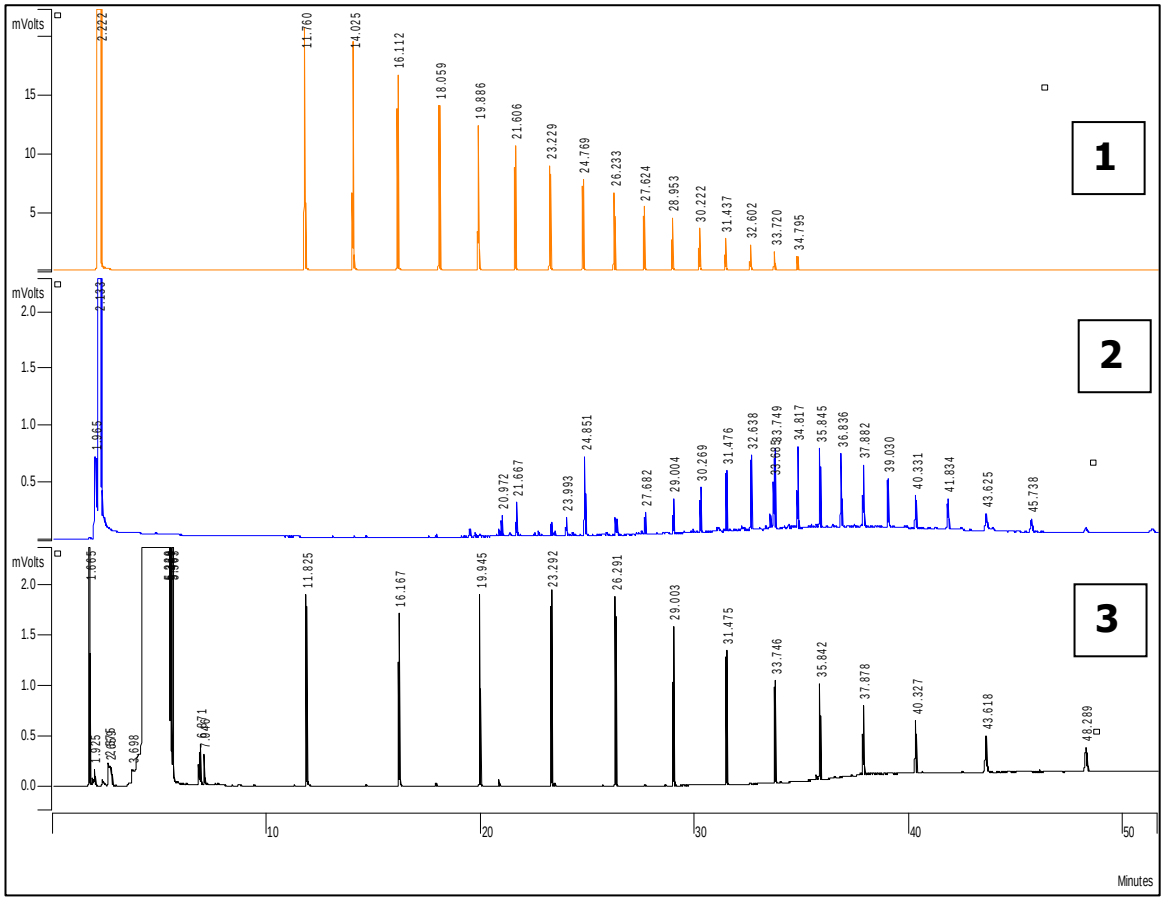


Figure 5.9 Overlaid chromatograms of (1) DRO standard mix (2) Sample 3.1 (3) Alkane standard mix.

The tested sample 3.1 (chromatogram 2) did not elute until 20 minutes at about C14 and no signs of volatiles (BTEX) at C5 –C9. Compounds in chromatograms 1 & 2 were prepared in DCM while chromatogram 3 was prepared in hexane as evident in the solvent elution peaks at C5-C9. If hexane or toluene or any hydrocarbon was used as solvent, the peaks within C5-C9 in chromatogram 3 would have been misconstrued. The basis of utilizing the reference standards for identifying the compounds in the samples was established.

The chromatograms of the hydrocarbon standards demonstrated that efficient separation and resolution was achieved by the Varian BV CP 3800 gas chromatograph with the chosen column.

5.6 Evaluation of total Petroleum Hydrocarbon Contamination in spilled soil samples.

The analysis of all the samples (47 samples) were carried out along with the standards as explained in chapter 4.5. Each sample point/hole yielded three samples taken at different depths, i.e. 15 cm (top), 15-30 cm (middle) and 30-60 cm (bottom) (refer to table 5.6) below. For instance, A1, A2, A3 etc stand for samples 1, 2, 3 etc taken at those spots and comprising of three depths each, such that chromatograms A1.1 represented top soil at 15 cm depth, A1.2 for middle/subsoil at 30 cm depth and A1.3 for depth of 60 cm of sample A1. Similarly, the same system was adopted for samples A2 – A12 and the control samples.

The average peak values of all the samples were recorded and their standard deviation and % RSD calculated at 95% confidence level (refer to Appendix 111 A.

Table 5.6 shows the statistical summary of the average concentration of the total petroleum hydrocarbons (TPH) in the analyzed oil-spilled soils, computed as shown in chapter 4.6.

The optimized method was applied to samples and standards as well. The Varian column was only able to separate hydrocarbons up to C34. The column had a maximum operating (programmed) temperature of 350°C and the limitation was that separation was only achieved up to 310°C. The split ratio of 1:25 was employed to overcome the possibility of column overload even as 1.0 µl was the injection volume applied for both standards and samples.

The standard deviations obtained for the retention time for each standard (figures 5.4 and 5.5), showed that the reproducibility of the results was very good. This also indicated that a reasonable and acceptable degree of precision was established in the results.

Table 5.6 A summary of average Total Hydrocarbon content (mgkg^{-1}) in all samples and controls.

SITE 1 A	No	SAMPLE MASS (g)			*AVERAGE PEAK AREA (mV*sec)			HYDROCARBON CONTENT (mgkg^{-1}) or (ppm)		
		TOP	MIDDLE	BOTTOM	TOP	MIDDLE	BOTTOM	TOP	MIDDLE	BOTTOM
A1	3	10.16	10.00	10.06	546	889	439	94±25	156±26	76±39
A2	2	10.49	10.25	**	797	388	**	133±38	66±19	**
A3	3	10.00	10.10	10.00	1343	1697	933	235±5	294±16	163±15
A4	3	10.01	10.11	10.02	443	482	482	77±44	84±18	84±14
A5	3	10.10	10.30	10.00	605	515	560	105±11	88±4	98±3
A6	3	10.08	10.00	10.00	54	48	45	9 ±7	8 ±1	7 ±1
A7	3	10.00	10.02	10.01	45	51	46	10 ±2	9 ±1	8 ±2
A8	3	10.02	10.39	10.48	259	323	345	45 ±11	54 ±7	58 ±13
SITE 1B										
A9	3	10.13	10.10	NA	248	216	NA	43 ±18	37±6	NA
A10	3	10.01	10.02	NA	269	202	NA	47 ±10	34 ±7	NA
A11	3	10.08	10.02	NA	1668	1820	NA	289±15	318±4	NA
A12	3	10.00	10.00	10.14	283	216	287	50 ±13	38 ±4	49 ±3
SITE 2	CONTROLS									
A12 X	3	10.11	10.02	10.10	0.0	0.0	0.0	0.0	0.0	0.0
AX13	3	10.01	10.11	10.00	0.0	0.0	0.0	0.0	0.0	0.0
AX1	3	10.20	10.14	10.11	0.0	0.0	0.0	0.0	0.0	0.0
AX2	3	10.00	10.12	10.13	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL	47									

* Average of three replicate analysis
** Not sampled due to impervious rock obstruction
NA =

Identification and quantification of the samples was based on the comparison of the chromatographic data with the reference standards (alkane and DRO mix) and the sample as shown in figure 5.10.

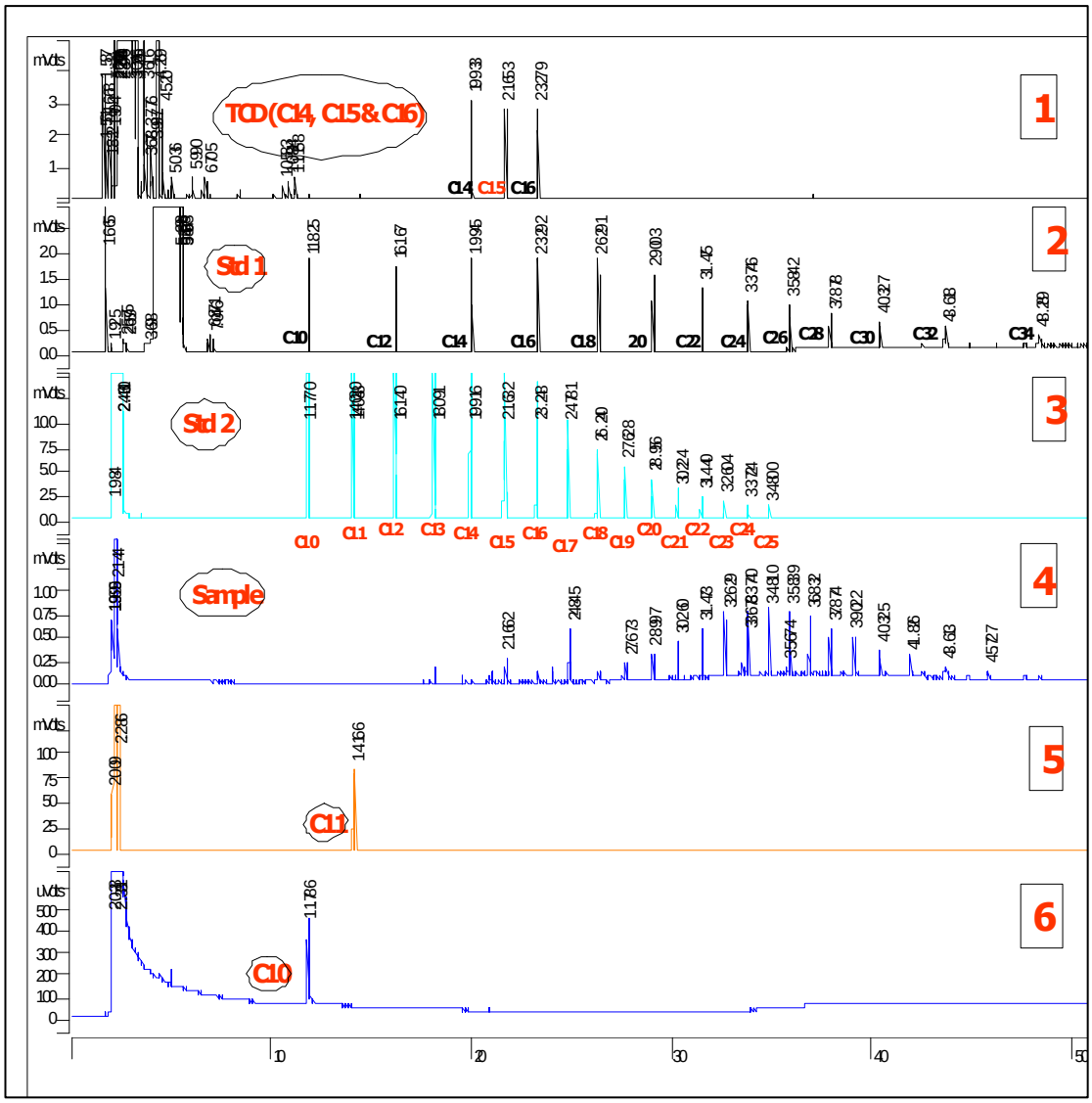


Figure 5.10 **Sample 3.1 (chromatogram 4) was compared with the reference standards (chromatograms 2 & 3) and other lab standards (chromatograms 1, 5 and 6) for confirmation of eluted peaks in samples**

Standards in chromatograms 3, 4, 5 and 6 above were prepared with DCM which was also the solvent with which the samples were prepared while Alkane and TCD standards were prepared in hexane and heptane respectively thereby showing evidence of peak contamination at BTEX retention time window as

shown in figure 5.11. However, the chromatograms of all the samples did not indicate BTEX at RTW C3-C9. Refer to chromatograms in Appendix IV.

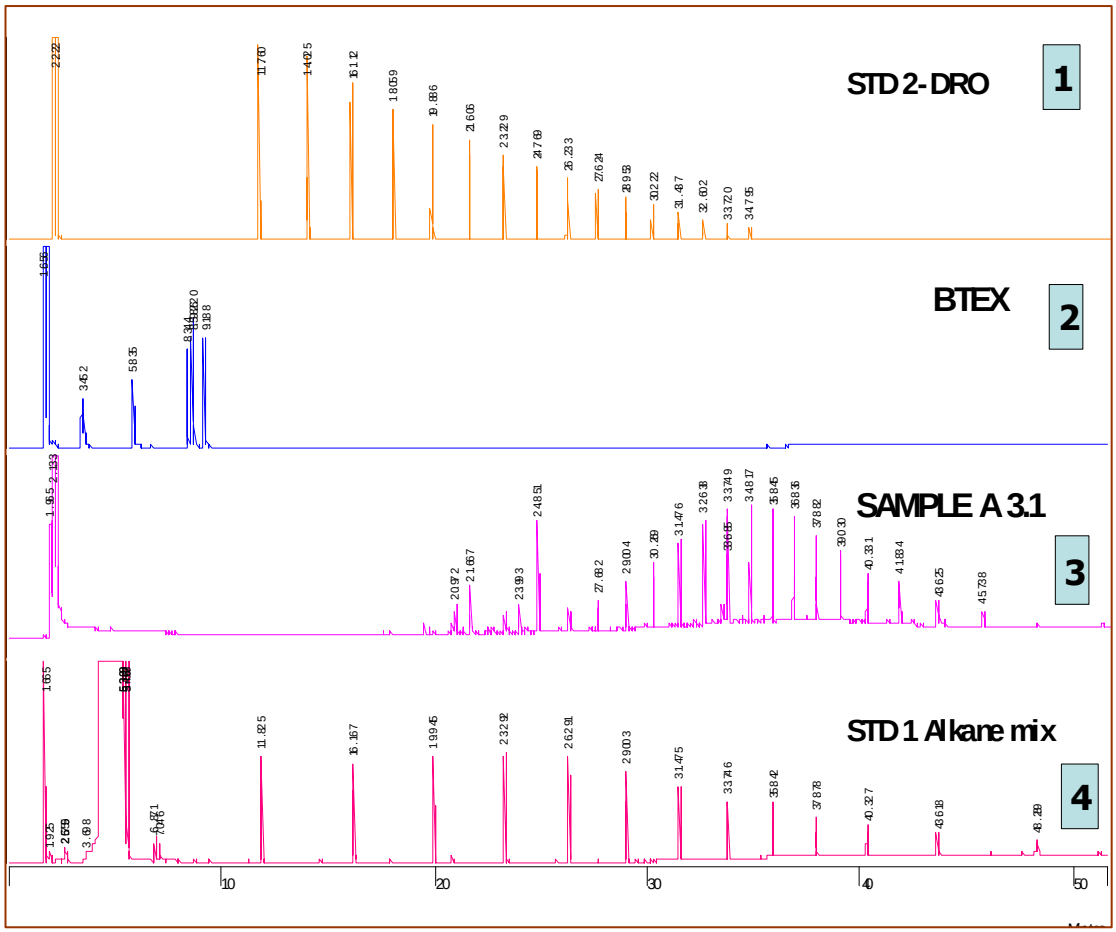


Figure 5.11 **Chromatograms of the sample (3) overlaid with two alkane reference standards (alkane and DRO mix) and BTEX standard.**

In the above figure sample A3.1 was used to represent other samples because it showed the widest spread of the contaminants in the optimized retention time window with the last compound eluting at 45.73 minutes while alkane reference standard covered up to C34 with 48.78 minutes retention time.

All the contaminants qualitatively identified and quantitatively validated fell within C10 – C34 and are named with their retention times in table 5.5

However, for purpose of classification C10–C26 (Diesel range organics, DRO) and C26-C34 (Lubricating /Waste Oil Organics WOO) are distinguished, while Gasoline Range Organics (GRO, encompassing BTEX) were not detected at the final sample analysis. The non detection of elevated levels of BTEX peaks in the sample could be attributed to their solubility in soil water, atmospheric temperature, type and extent of contamination [9] and evaporation [10] of the light crude oil on exposure for long time before sampling and analysis. This result is consistent with [11] that very low concentrations of organic pollutants (gasoline components) were found in soil and water after Katrina.

Control samples from similar geographical non-spilled areas, randomly collected and analyzed as the standards along side with other samples did not contain any petroleum hydrocarbons (see chromatograms in figure 5.12). Only the peaks of the extracting solvent, DCM were seen at 2.13 minute in all the control samples.

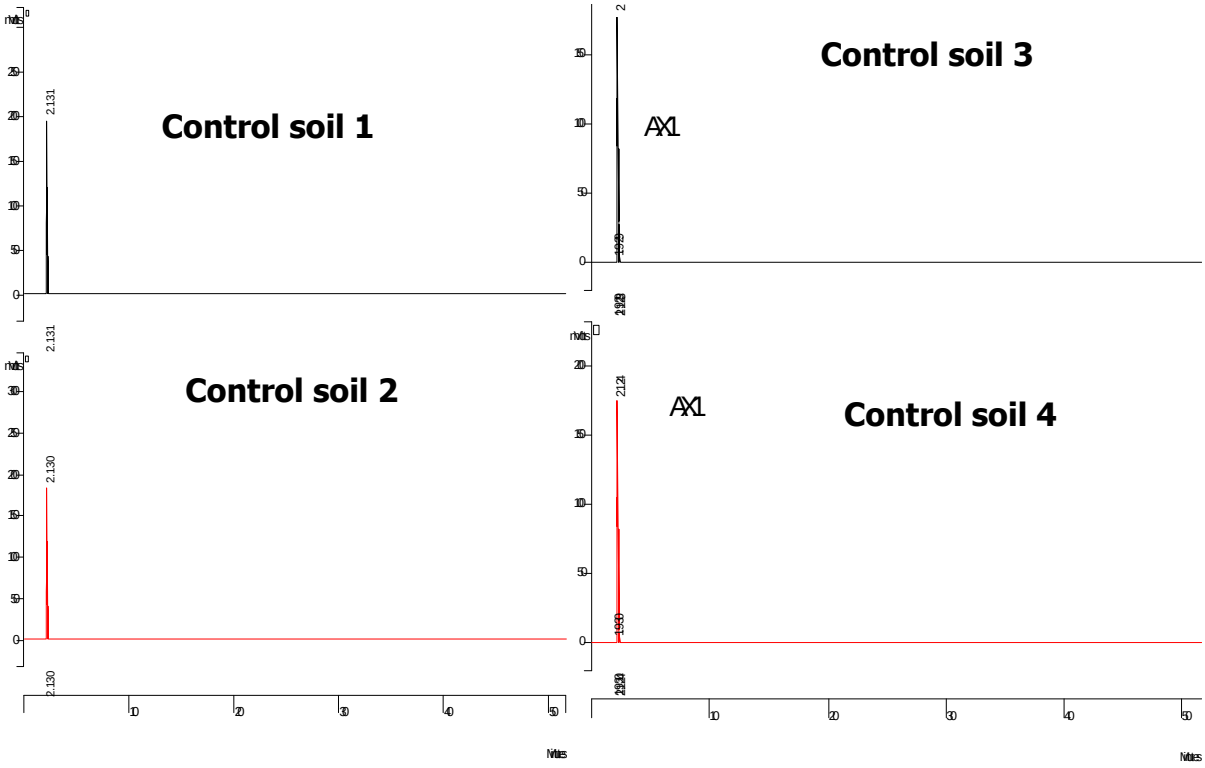


Figure 5.12 **Chromatograms of four control soils. Only the solvent peak came out at retention time of 2.13 minutes with no evidence of possible contamination with hydrocarbon contaminants over the run time**

In table 5.6, the TPH concentration for the top soils (15 cm depth) range from (9 ±7 to 289 ±15) $mgkg^{-1}$. The middle or sub-soils (30 cm depth) had a concentration range of (8 ±1 to 318 ±4) $mgkg^{-1}$ and a range of (7±1 to 163 ±15) $mgkg^{-1}$ was recorded for the 60 cm depths measured. The overall level of TPH recorded here in the petroleum contaminated site ranged from (7±1-318±4) $mgkg^{-1}$. Site A3 and A11 had high TPH concentration with the middle soil (15-30 cm depth) and sample A11 having the highest value of TPH (314±4 $mgkg^{-1}$) followed by 294±16 $mgkg^{-1}$ at the same level in sample A3.1

The lowest depths (60 cm) in most of the samples recorded had significantly low value of TPH though concentrations of total petroleum hydrocarbons did not decrease generally with depth as pointed out by [12, 13]. The concentration of TPH at the middle/sub-soil (15-30 cm) depth was higher than the concentration range reported by [12, 14, 15, 16] for oil spilled soils of other parts of Niger Delta. However, no significant level of TPH was recorded for the control soil samples taken from similar geographical non-spilled areas. The samples showed elevated concentrations of TPHs when compared with control samples in all the sites. The high levels of total petroleum hydrocarbon contamination observed in this study for spilled soils are comparable to with levels obtained by [13, 17, 18] and far exceeded the fifty parts per million (50 $mgkg^{-1}$ or ppm) compliance baseline limit [19] set for petroleum industries in Nigeria.

5.7 Assessment of Penetration capability of the Hydrocarbon Contaminants

In chapter 2, it was reported that the concentration levels of hydrocarbons present in contaminated site pose a health risk to humans, plants and animal lives. It becomes imperative to assess the type of hydrocarbons and the extent of depth penetration for the purpose of remediation action and record. Therefore this section of the study characterized the hydrocarbons into groups based on their degree of penetration within the soil strata. Basically, three

major groups of petroleum hydrocarbons are known, classed and adopted in this work. These are:

(i) The Gasoline Range Hydrocarbons (GRO), generally eluting in window C5-C9.

(ii) The Diesel Range Organics (DRO) elutes from C10-C24 or C26.

(iii) The Lubricating or Waste Oil Organics (WOO), eluting above C26.

The concentrations of GRO (BTEX range) in the analyzed samples were insignificant; nevertheless, the presence and concentrations of C10-C26 and C26 & above had been identified and quantified. The penetration, percentage distribution and migration of these groups of hydrocarbons in the samples are considered below. However, for the purpose of this assessment, the site samples were classified into three groups based on the observed pattern of hydrocarbon penetration and distribution.

Group 1 contains six (6) samples/holes (A1, A3, A5, A8, A10 & A11), each comprising of three (3) sampling depths (18 sampling depths in all). This group had competitive percentage depth penetration for both DRO (C10-C26) and WOO (C26 & above) as shown in Figures 5.13-5.18, with DRO taking advantage.

Group 2 has three (3) samples (A6, A9 and A12) with nine (9) sampling depths. In this group, 100% presence of DRO was found in all the soil depths with no contribution from WOO (refer to graphs in figures 5.19-5.21).

There are 3 samples (A2, A4 & A7) with 9 sampling depths forming group 3. This group had DRO dominating the soil depths with little contribution from the Lubricating or waste oil hydrocarbon range - C26 & above (see figures 5.22-5.24).

In the graphical representation in figures 5.13-5.24, each sample hole yielded three samples taken at different depths of 15 cm (top), 30 cm (middle) and 60 cm (bottom). The chromatogram for the top soil (15 cm depth) is successively followed by chromatograms for the middle sample (30 cm) and the greatest measured depth (bottom, 60 cm). The chromatograms for each sample are represented with a bar graph side by side showing penetration and distribution of hydrocarbons in the range C10 - C20 and C21 - C34.

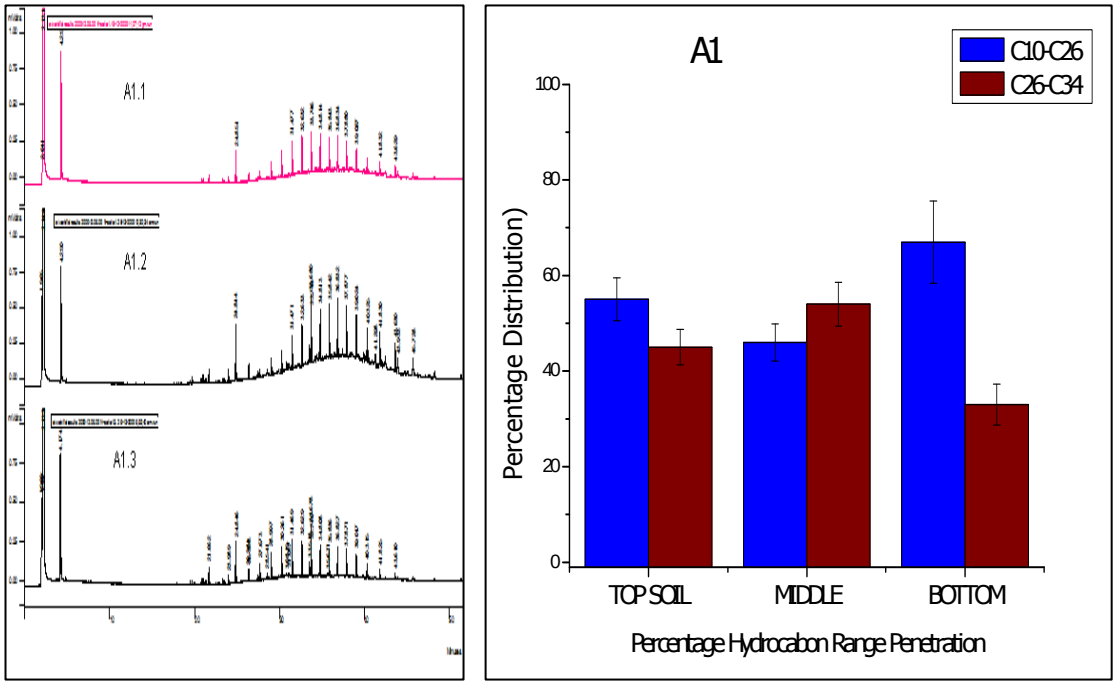


Figure 5.13. **Chromatograms of sample A1 showing the hydrocarbon range found in top (A1.1), middle (A1.2) and bottom (A1.3) soil levels.**

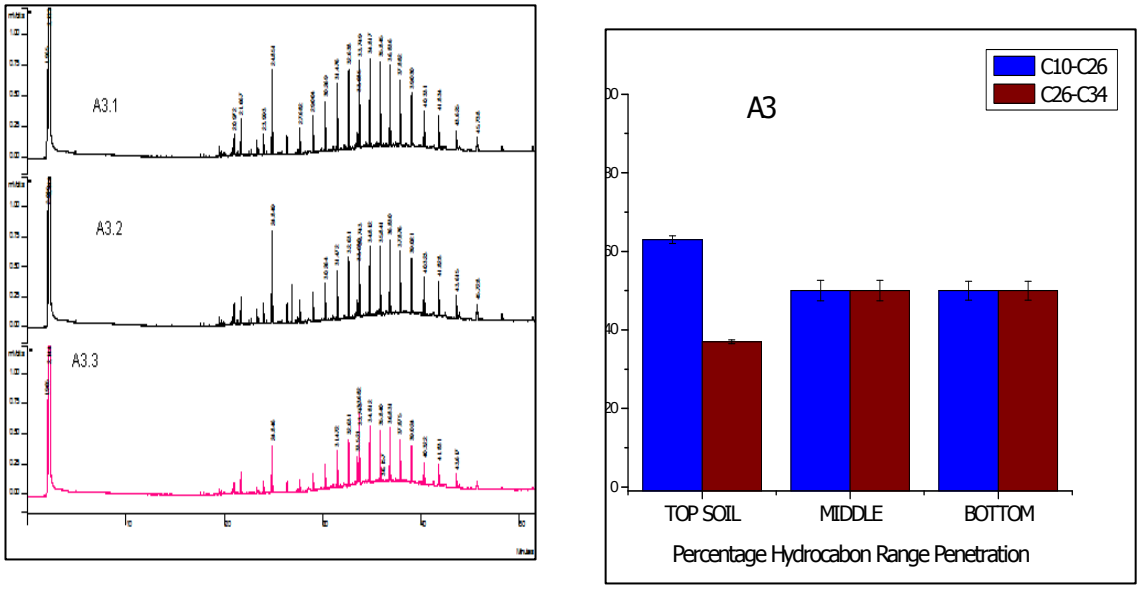


Figure 5.14 **Chromatograms of sample A3 showing the hydrocarbon range found in top (A3.1), middle (A3.2) and bottom (A3.3) soil layers.**

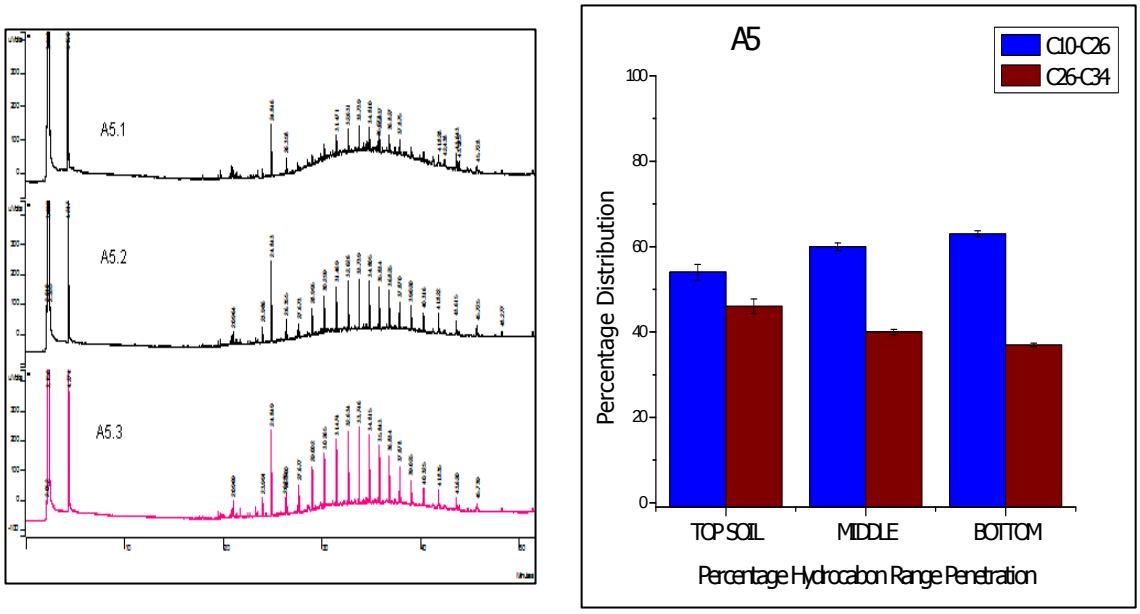


Figure 5.15 **Chromatograms of sample A5 showing the hydrocarbon range found in top (A5.1), middle (A5.2) and bottom (A5.3) layers.**

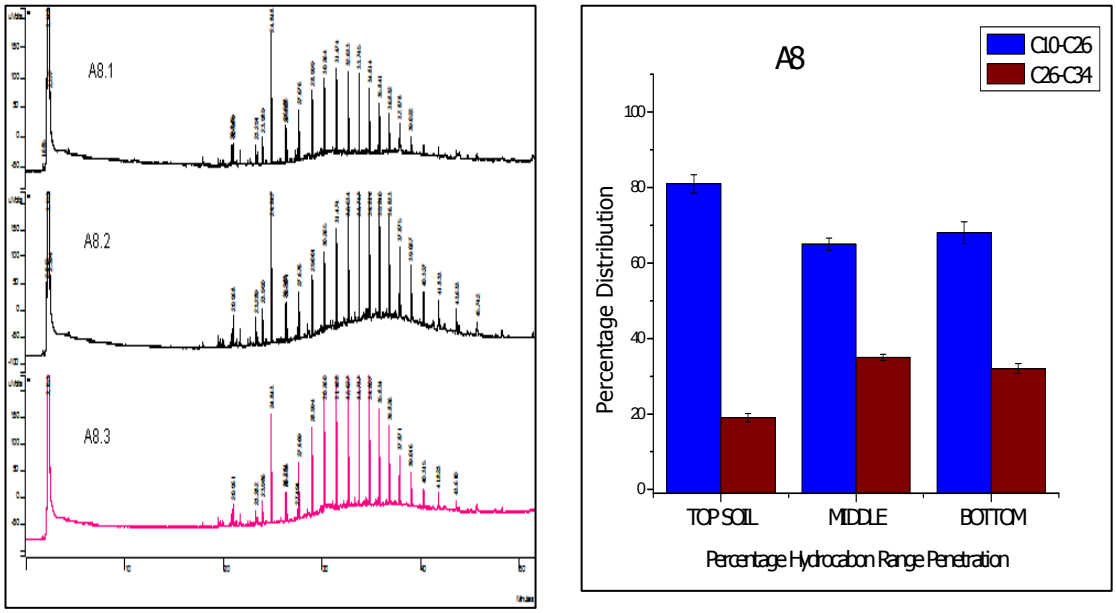


Figure 5.16 **Chromatograms of sample A8 showing the hydrocarbon range found in top (A8.1), middle (A8.2) and bottom (A8.3) levels.**

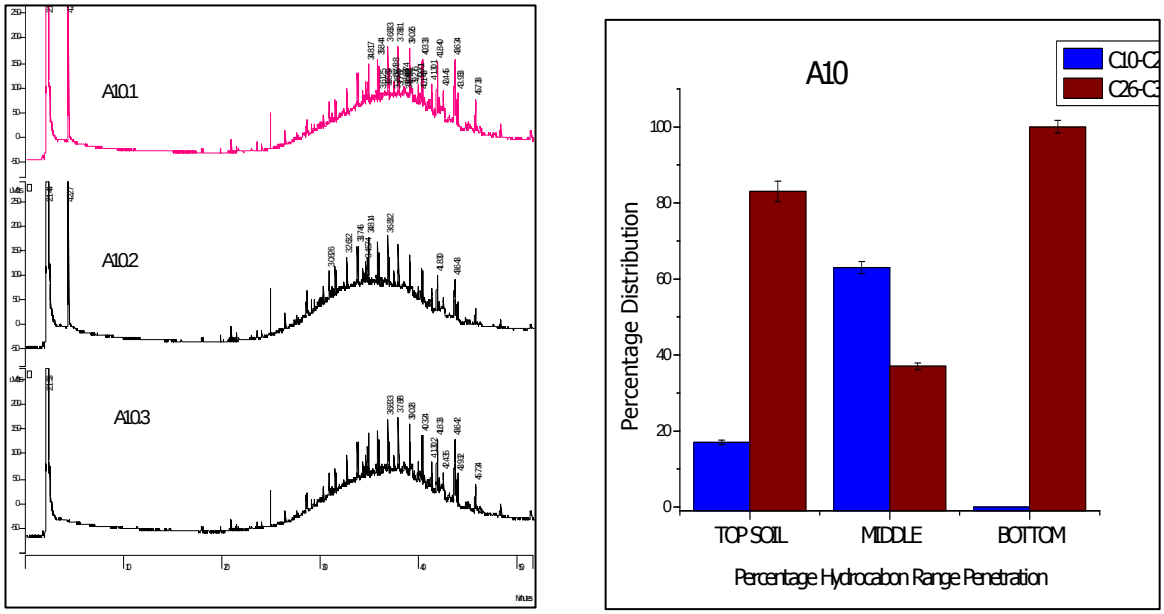


Figure 5.17 **Chromatograms of sample A10 showing the hydrocarbon range found in top (A10.1), middle (A10.2) and bottom (A10.3) levels.**

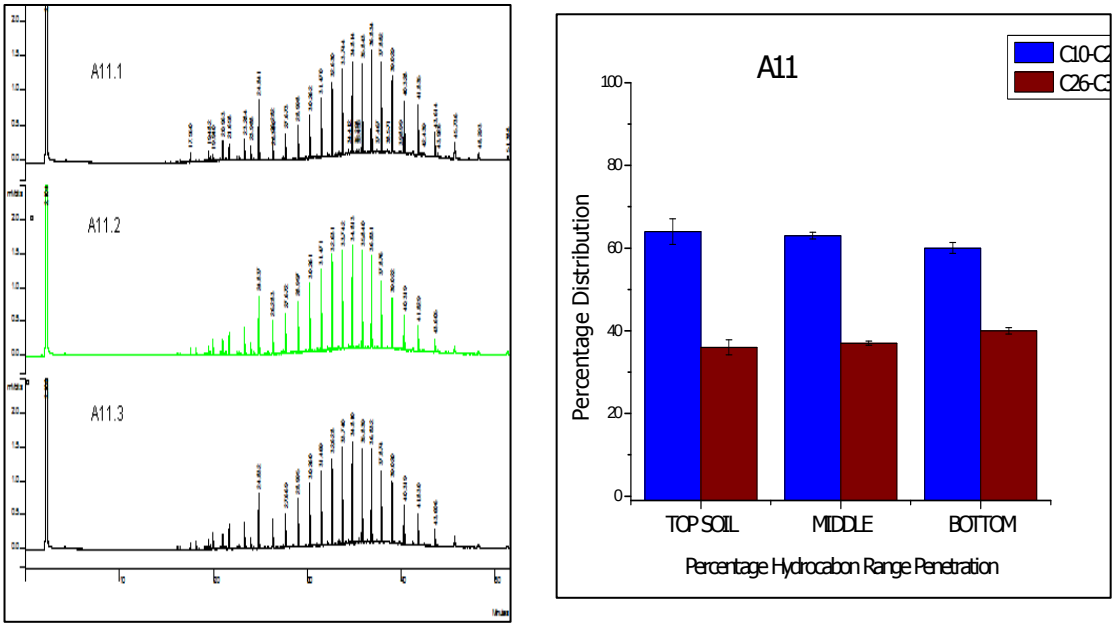


Figure 5.18 **Chromatograms of sample A11 showing the hydrocarbon range found in top (A11.1), middle (A11.2) and bottom (A11.3) soil strata.**

Figure 5.13 shows the penetration, percentage distribution bar graph and chromatograms of sample A1 placed side by side. The chromatograms are overlaid from top soil -15 cm (A1.1) to middle level, 30 cm (A1.2 to last level

measure – 60 cm (A1.3). The hydrocarbon contaminants in the top soil comprise of 55% DRO and 45% WOO at 15 cm depth. At 30 cm depth, middle soil, DRO and WOO presence was 46% and 54% respectively. 67% DRO and 33% WOO were distributed at 60 cm being the deepest depth measured.

The presence of DRO at top soil in sample A3 was 63% and 33% WOO.

50% presence was recorded for DRO and WOO in sub-soil and bottom levels of sample A3.

Sample A5 in figure 5.15, had 54%, 60% and 63% DRO for top to bottom level while WOO had 46%, 40% and 37% presence from top to bottom. The distribution of hydrocarbon contaminants in sample A8 indicated that DRO had higher percentage of 81, 65 and 68 top to bottom levels while WOO are less.

Figure 5.18 representing sample A11 had greater percentage of DRO in all the soil levels than the percentage penetration of the WOO.

Sample A10, collected at the Well Head (Chapter 4, section 4.2.1, figure 4.3) had a peculiar percentage distribution and depth penetration pattern. 100% WOO was found to have penetrated to bottom level of 60 cm and no DRO, while the top soil also was rented with more than 80% WOO.

In group 2 of soil samples shown in figures 5.19 – 5.21, the chromatograms and the bar graphs showed 100% penetration and distribution of DRO at all the soil levels from top to bottom. There was no detection of the presence of WOO in these soil samples.

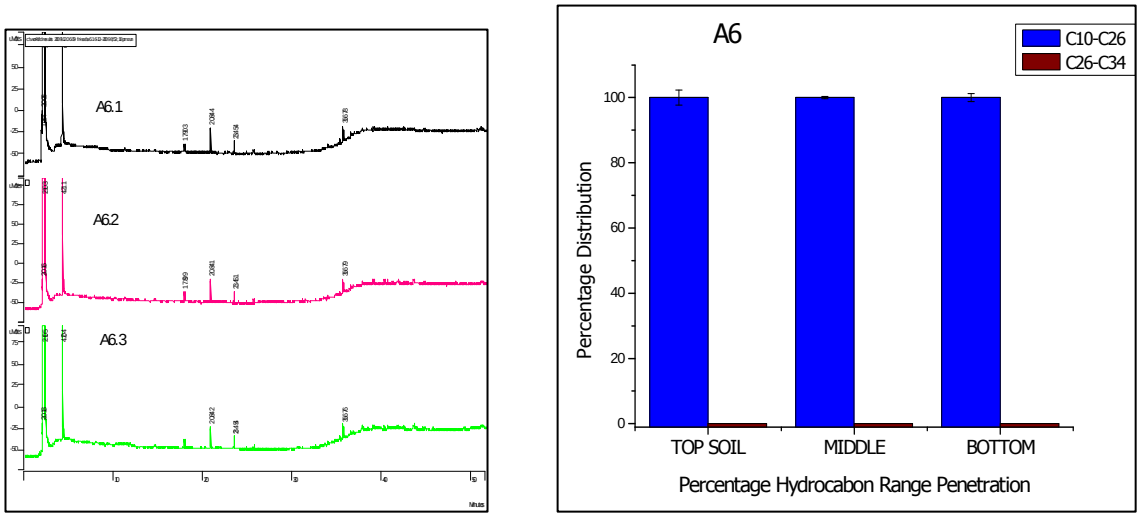
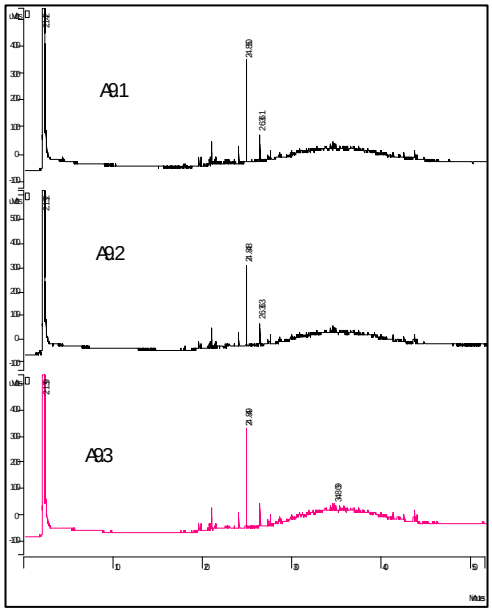


Figure 5.19 **Chromatograms of sample A6 showing the hydrocarbon range found in top, middle and bottom soil levels on a bar graph.**



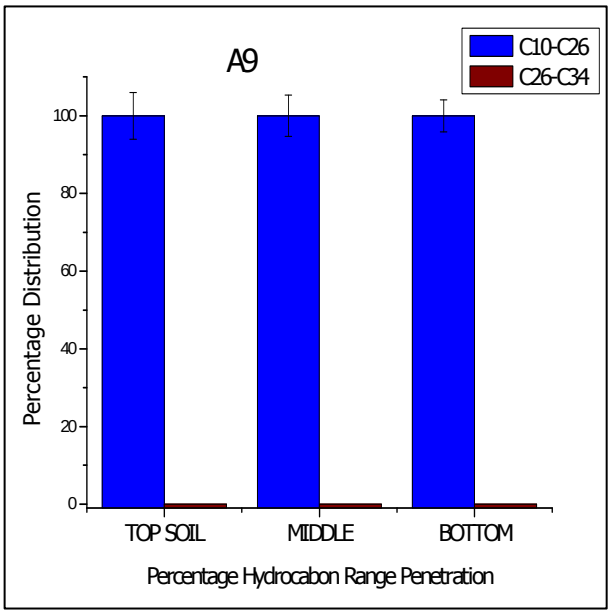
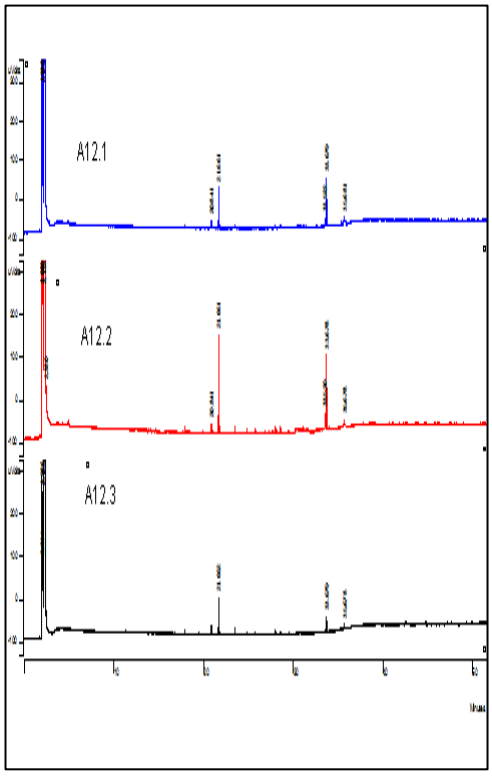


Figure 5.20 **Chromatograms of sample A9 showing the hydrocarbon range found in top, middle and bottom levels on the bar graph at the side.**



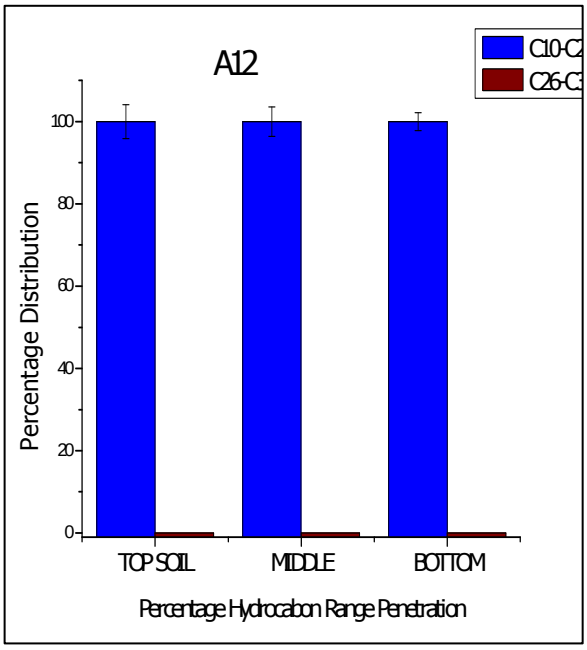


Figure 5.21 **Chromatograms of sample A12 showing the graph of the hydrocarbon range found in top, middle and bottom strata.**

The dominance of the diesel range organics (DRO) in these sites gave an insight into the type of remediation plan to be adopted and application of soil treatment methods that could be applied.

The percentage penetration and distribution of petroleum hydrocarbons were crucial in Samples A2, A4 and A7 in group 3 (refer to figures 5.22-5.24 below) in C10 –C26 hydrocarbons significantly prevailed.

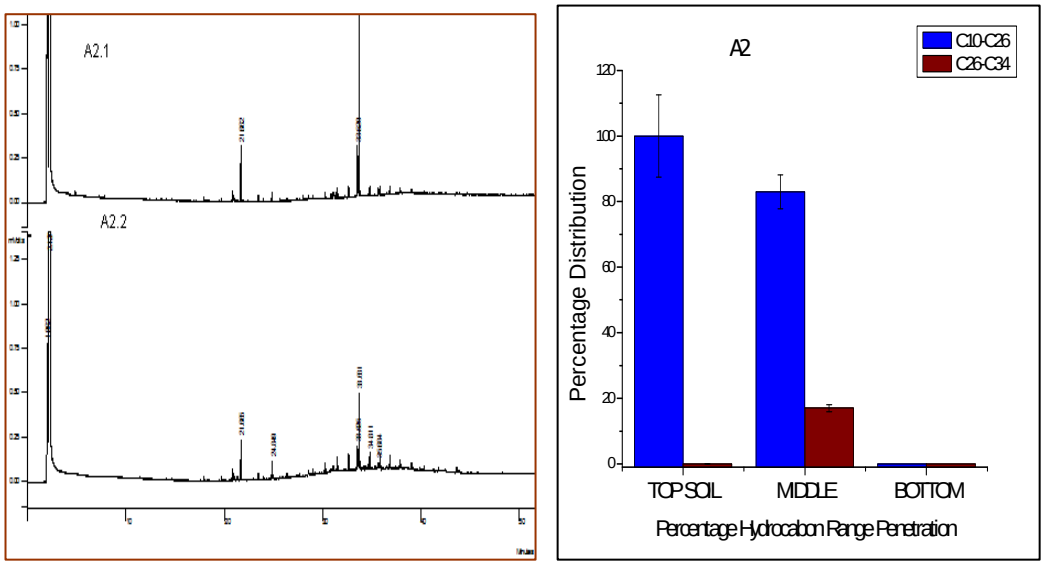


Figure 5.22 **Chromatograms of sample A2 indicating the bar graph of the hydrocarbon range found in top, middle and bottom soil strata.**

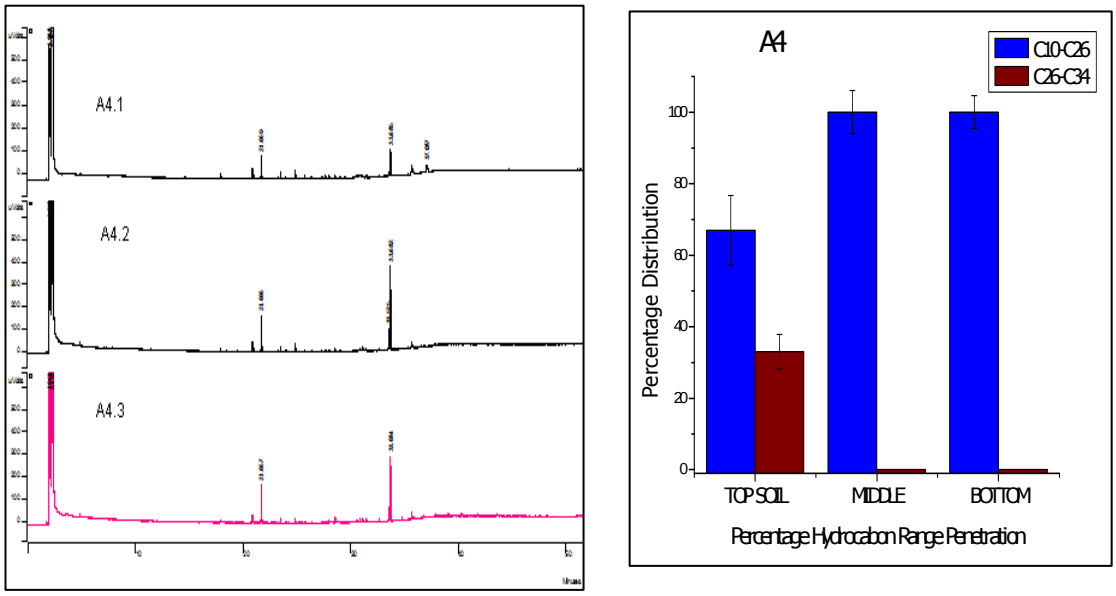


Figure 5.23 **Chromatograms of sample A4 representing the hydrocarbon range found in top, middle and bottom soil levels.**

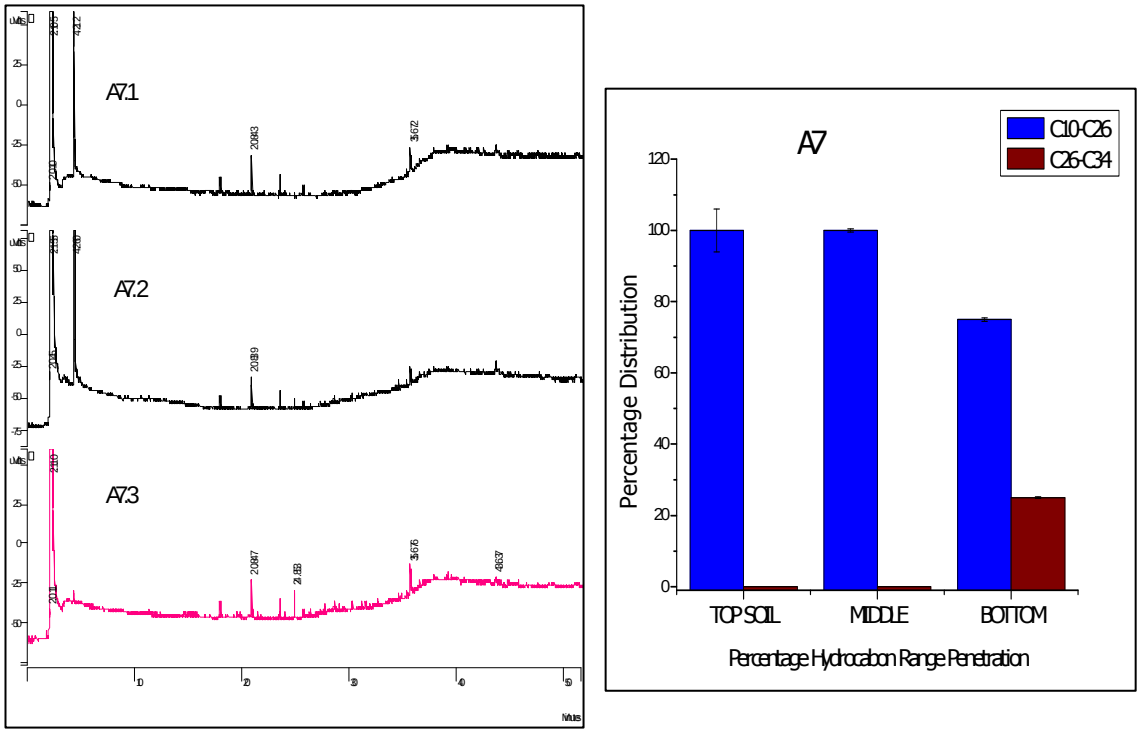


Figure 5.24 **Chromatograms of sample A7 displaying the bar graph of hydrocarbon range found in top, middle and bottom soil strata.**

In sample A2, figure 5.22, 60 cm depth was not sampled due to impervious rock obstruction. Top and middle soil levels had 100% and 83% of DRO (C10-C26) respectively. Middle and bottom levels of sample A4 had their contamination full with 100% DRO (C10-C26) hydrocarbon range.

5.8 Chemometric Evaluation of the samples Characteristics

In order to optimally extract useful information content from the analyzed chromatograms, a supplicated Chemometric technique was employed. The mathematical analysis often exploits the use of software. MINITAB^{TM} software was used in this work. Principal Component Analysis (PCA) was adopted to interpret and classify chemical characteristics of the sample by Cluster analysis (Refer to Minitab data information in Appendix V A-C).

The aim was to fuse the huge chromatographic data into a simple line or plane graph projection, thereby reducing the amount of data or number of

dimensions without losing the integrity and relevant information of the samples [20]. PCA used combined concentration and sample-discrete-identity information while related techniques like Principal Component Regression (PCR) and Partial Least Square (PLS) could only limit its quantification on concentration parameter. Average sample peak areas were normalized and transposed using minitab. PCA computed the correlation and covariance matrices to establish the principle components of all samples.

Principal component one and two (PC1 and PC2) were plotted. Display plots judging the different principal components were used to examine the scores of the first two principal components [21] (refer to chapter 5, section 5.8).

A dendrogram showing similar distance and characteristics of the individual samples was plotted in excel. (Refer to figure 5.28).

The sampling and extraction procedures are explained. GC-FID method development and optimization was achieved. The instrument was checked, calibrated with RTW and other reference standards with the corresponding analysis of the samples.

Cluster observation analysis was applied to all the samples level using TPH as a parameter to assess the chemical similarities and or otherwise of the contaminants. Minitab software -Eigen value (**Scree**) plot displayed Eigen value profiles associated with a principal component versus the number of components as seen in figure 5.25.

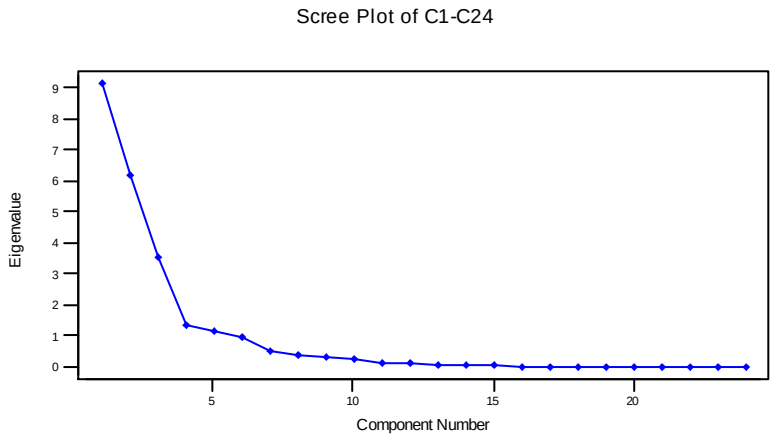


Figure 5.25 PCA Scree plots of the Eigen value and the principal components

A **score** plot was carried out to check the scores for the second principal component (**y-axis**) versus the scores for the first principal component (**x-**

axis) and values for all samples as shown in figure 5.26 (Refer to Minitab data information in Appendix V A)

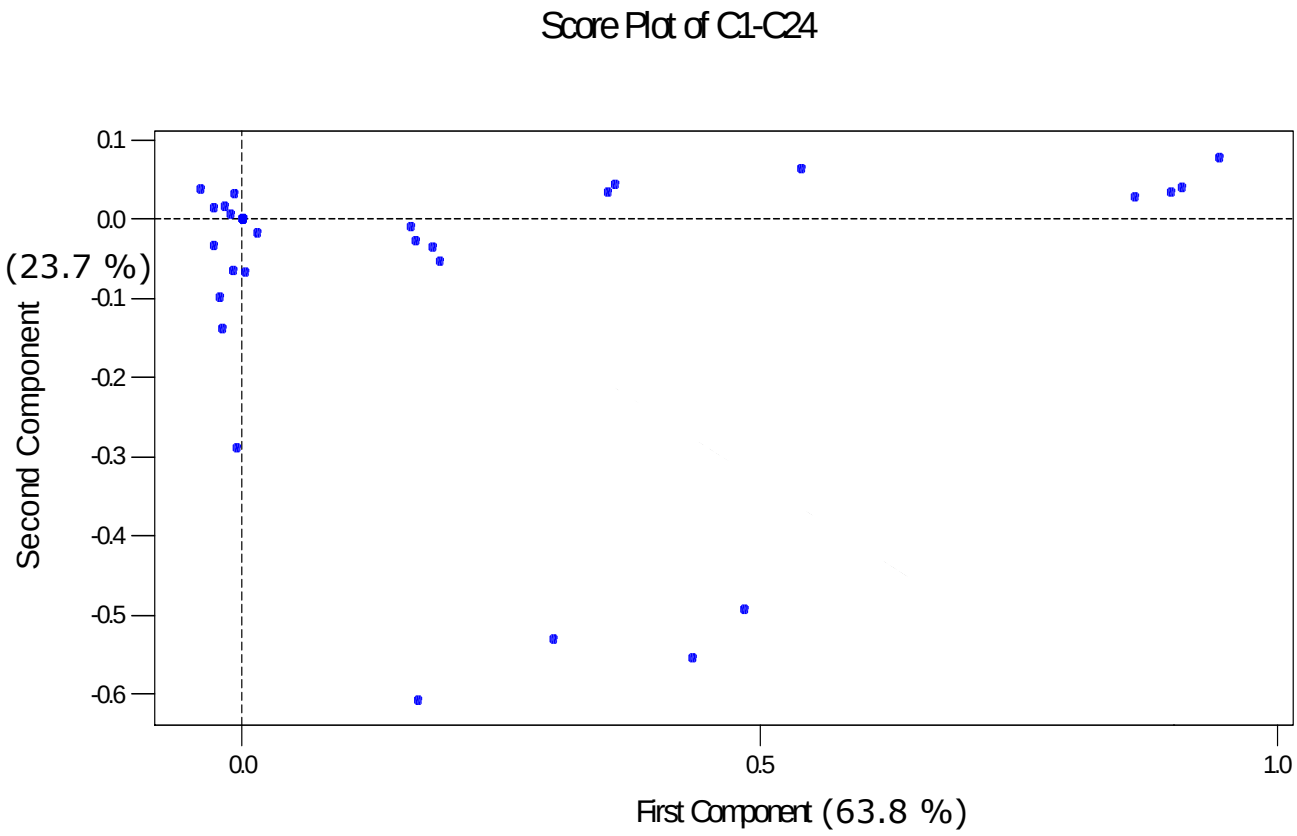


Figure 5.26 **Score plot of PC1 (63.8 % variance) and PC2 (23.7 % variance) of all samples.**

The score plot gave the information of every sample point and the three levels of sampling except sample A12 (refer to Appendix VB for data information from Minitab software) Each dot represents one sample level and 11 samples have 33 dots for the three depths of top, middle and bottom levels.

Cluster analysis was achieved by using sample information in PC1 and PC2 and plotted in Microsoft excel. The goal was to identify exact hydrocarbons with similar chemical characteristics. Clustering of observations was applied with the complete linkage method, squared Euclidean distance, and standardization to bring out the different clusters. The data was transposed into excel to spell out the individual sample and their depths. Figure 5.27 characterized the samples into groups of chemical similarity (Refer to Minitab data information in Appendix V B)

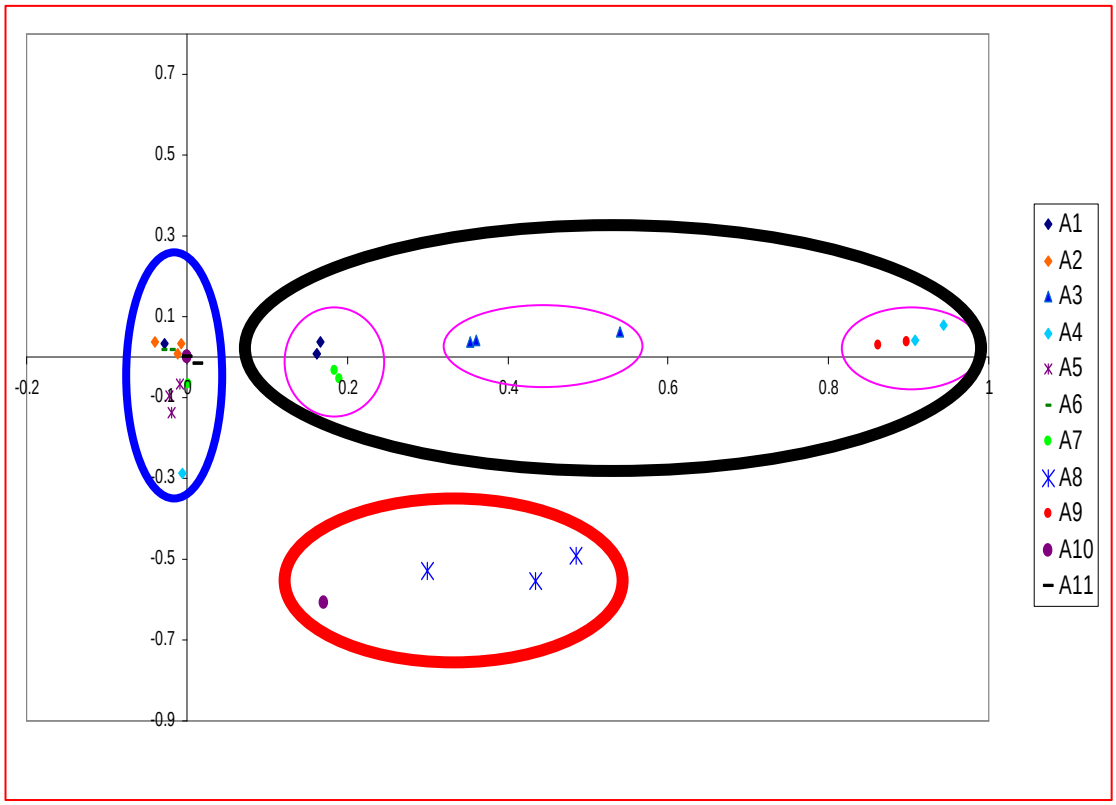


Figure 5.27 **Cluster pattern for samples. Three main clusters were identified as represented with red, black and blue circles. The samples represented in the circles are shown in the legend**

In figure 5.27, three main clusters were identified; namely, the clusters marked with red, black and blue circles. The clusters attempt to classify the samples into similar chemical characteristics without losing their integrity. Samples A8 and A10 in the red circle were classified as being chemically similar. This is in agreement with pattern of distribution of the hydrocarbons obtained in figures 5.16 and 5.17. The black circle formed close clusters of sample A1and A3 to be similar in characteristics as in group 1 classification of the types of hydrocarbon penetration in soil level in figures 5.13 & 5.14 above. Samples A7, A2 and A4 are similarly grouped together. This constitutes group 3 of similar hydrocarbons present in soil depths in figures 5.22-5.24.

The cluster observation of chemically similar samples was also displayed by the group in blue circle. In this group, sample A11 & A5 were patterned alike while sample A6 and A9 formed the same cluster (see section 5.7). A dendrogram (figure 5.28) with distance and similarity characteristics of the individual

samples was plotted using Minitab software as shown in the amalgamated information table (refer to Appendix V B & V C).

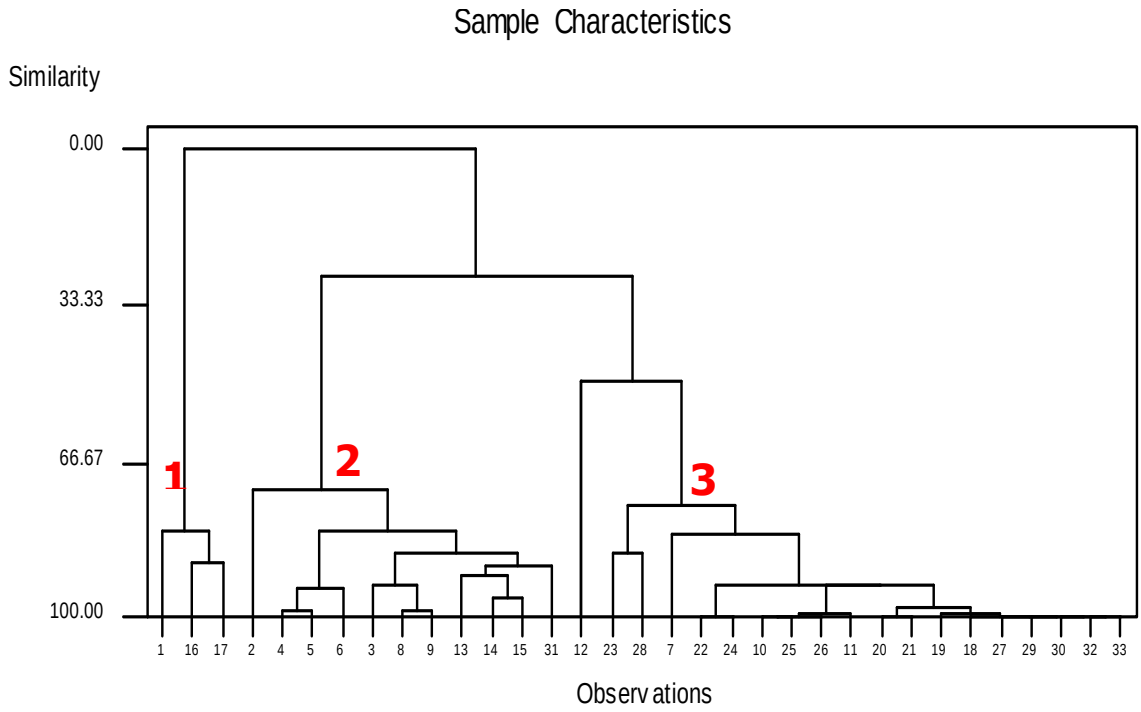


Figure 5.28 **Dendrogram analyses of TPH cluster observations and variables in Niger Delta spilled soil.**

In figure 5.28, the dendrogram cluster analysis of the oil-spilled soils was analyzed using TPH variables. The high resolution dendrogram graph identified three similar groups inherent in samples as previously established in section 5.7. The dendrogram had data information for three depths of 11 sampling points amounting to 33 samples as shown on the x-axis of the dendrogram. Numbers 1–33 represent sample A1–A11 (three levels each). On x-axis, numbers 1, 2, 3, stand for sample A1 (top, middle & bottom), 4, 5, 6 ….and 31, 32 & 33 stand for samples A2 and A11 respectively for all the 3 levels.

Cluster 1 had sample A6 with only DRO (C10-C26) hydrocarbon contaminants, Cluster 2 comprised chemically similar group of samples A2, A3 and A5 with mixed DRO and WOO contaminants. This is in conformity with the results obtained in section 5.7 for the types of TPH present in the soil levels. Samples A8, A10, A11 and A4 and A9 formed cluster 3 of the dendrogram. Sample A1

for no reasons had independent cluster because A1.1 (top), A1.2 (middle) and A1.3 (bottom) formed outliers from the three clusters.

Cluster observation analysis of the soil has been able to classify the TPHs present in the different soil levels. This information can also be useful in identification of the source of crude oil spillage based on the proportionate presence of organic contaminants and the classification of organic contaminants having similar chemical characteristics [22]. The TPH distribution and classification patterns and will offer a useful tool for bioremediation process.

5.9 Conclusion

A method for the analysis of soils spilled with crude oil was developed and optimized. The results of the analysis of the soil and controlled samples were based on the optimized method. Sample extraction procedures, optimum extraction time and successful sample clean-up were also undertaken concisely.

The results of analysis of such samples in this chapter revealed that the TPH concentration in all the levels of soil strata measured ranged from 7±1-318±4 $mgkg^{-1}$. This work also expressed the relative concentration differences between sampling points. BTEX range of volatile organics were not detected in this analysis partly due to its reduced concentration resulting from evaporation after prolonged exposure of spill before sampling was undertaken. The preliminary method developed for the BTEX did not detect any BTEX below 5 ppm ($mgkg^{-1}$)

The concentrations and penetration ranges for two groups of TPHs were shown for C10-C26 (Diesel Range Organics) and C26-C34 (Waste Oil Organics), with DRO found occurring in almost all depths in the sampling site.

Cluster observation analysis was applied to reveal not only the nature but chemical similarity of the oil contaminants in all the levels as they penetrated.

This work showed types, distribution and migration pattern, penetration levels of the petroleum hydrocarbon contaminants in the study area. This information is vital to the State Government (Ministry of Environment and Natural Resources) and the oil Industries for monitoring further spills and largely to plan for a suitable remediation activity. The results obtained from this work

portrayed that bioremediation would be the recommended choice of remediation.

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CHAPTER 6

CONCLUSIONS AND FURTHER WORK

6.1 Conclusion

Referring to chapter 1 section 1.6, the major aim of this work was to study, develop and implement validated and traceable chromatographic methodology for qualitative and quantitative assessment of petroleum contaminants in soils of Niger Delta under the tropical weather conditions. This was to be achieved by:

(i) Developing an optimized GC-FID method for the analysis of petroleum hydrocarbons and key biomarkers of crude oil contamination in soil such as Benzene, toluene, ethylbenzene and xylene (BTEX). A profile of these compounds and several Liquid Hydrocarbons was established in chapter 3.

(ii) Evaluating the concentration range of these contaminants in the spilled site

(iii) Comparing the penetration levels of hydrocarbon contamination at different depths at each location.

(iv) Exploring the possibilities of grouping the TPH contaminants into chemically similar characteristics based on Chemometric Cluster observation analysis.

This research has shown that it was possible to use the validated GC-FID method to qualitatively and quantitatively assess the TPH content in the crude oil spilled soils and also exploit the use of Chemometric Cluster analysis to group the hydrocarbons with similar chemical characteristics. The results could not provide conclusive evidence of either the presence or absence of contamination by liquid hydrocarbons beyond C34 (tetratriacontane). This was the limitation of the method. In realising these aims, remediation and possible

preventive measures will be recommended to the State Government and the Oil Industries.

Methods were developed and validated for GC-FID analysis of petroleum contaminated soil. The results showed a total petroleum hydrocarbon level of about 318±4 mg kg^{-1} in sample A11. This was observed at the middle sample range (30 cm depth). The lowest concentration occurred at the deepest levels measured (60 cm depth) with sample A6 at site 1A having a concentration of 7±1 $mgkg^{-1}$ while control sites had no detectable level of TPH contamination. The amount of petroleum hydrocarbon contamination found in the soil levels and the extent of penetration makes it necessary for the Government and the oil sectors to consider remediation measures since only good remediation practices will go a long way towards ensuring a safer environment for all.
A physicochemical study of all the sites is recommended to identify which remediation measures could be implemented based on the type and extent of contamination reported in this work.
The unacceptable risks [1] to human health and the environment resulting from the release of petroleum hydrocarbons to the soil, ecosystem, water resources, property and other environmental receptors should be urgently addressed and properly managed.

Despite limited information on the migration and depth penetration of hydrocarbons in soils, data from this study showed hydrocarbon concentrations and their penetration capability. There is also scarcity of data on Chemometric Cluster classification of TPHs, data recorded in this work attempted to classify the hydrocarbons penetration in the soil depths according to their chemical similarities thereby providing informative guidelines to the type of bioremediation procedures envisaged.

6.2 Critical Overview

All experimental work was carried out in the laboratory milieu under ambient temperature. Instrumental to the success of this research was the use of Hydrocarbon Standard Reference Materials to qualitatively and quantitatively

identify and validate the soil hydrocarbon contaminants. Clean up of sample extracts was crucial to the efficiency and reproducible performance of the column.

Collection of soil samples took place about 3 months after oil spill and during the dry season (soil temperature ≈ 38-40°C) in Niger Delta. This period of sampling has great implication on the on the evaporation of the volatile compounds (BTEX) as their measured concentrations were reduced minimally beyond the instrument detection limit. Evaporation may reduce the concentration of the volatile compounds but could concentrate some other constituents of the crude oil in soil.

No comparison was made to explore recent techniques of extraction (such as MAE, ASE, SFE) with the Soxhlet extraction technique used. This practical relative comparison would provide valuable information on the effectiveness and otherwise of the method.

Methods [2] could be developed to unravel the presence and levels of other contaminants not from petroleum products as the GC method adopted in this research was only limited to the identification and quantification of TPHs up to C34, tetratriacontane.

Maximum limits of TPH were not specifically stated as DPR [3] fixed the baseline limit at 50 $mgkg^{-1}$ (ppm.) in Nigeria. Heath et al [4] stated 100 $mgkg^{-1}$ as maximum level of TPH contamination in soils in United States. Soil Concentration Limits [5] was set at 100-400 $mgkg^{-1}$ for soil TPH/Diesel/ Gasoline in United Kingdom. A considerable amount of work has been published on the concentration range of TPHs in soils. These studies showed that about 1 to 100 $mgkg^{-1}$ of TPH contents were found in soils [6, 7, 8, 9]. In another study [10, 11, 12, 13], the TPH concentration in soil was reported between 100 to 500 $mgkg^{-1}$. Some publications [14, 15, 16, 17], reported the amount of TPH in soil to vary considerably to a few thousand $mgkg^{-1}$.

Soils contaminated with crude oil spills and other petroleum products are recurrent and pervasive problem occurring spontaneously in Niger Delta. However, a successful and meaningful cleanup of these contaminated soils is an all-round challenge. This study was limited to the sites located at Ikot Ada Udo

in Akwa Ibom State, South-South Niger Delta, Nigeria where all crude oil impacted soils were collected.

The need for more number of samples and deeper depths in subsequent analysis is rather important for comparative records. Sampling and analysis of different spill sites, including water and ambient air will provide extensive data information and application. The data obtained will be a useful guide not only to the oil industries, different arms of Government but on environmental health issues in the area.

6.3 Further Work

This work has shown the qualitative and quantitative evaluation of Total Petroleum Hydrocarbons (TPHs) in oil spilled land sites through the pathways of sampling, storage, preservation, extraction, extract clean-up and GC analysis. Undoubtedly, sample preparation in contrast to current advanced instruments is dependent on long established conventional techniques, sometimes boring, time and solvent consuming among possible introduction of contaminants. Recent advances in analysis strive to preclude or reduce this aspect minimally. Carrying out a reliable field sampling is crucial for the success of any environmental study. In order to minimise loss of analyte during sampling, transportation, storage, extraction and cleanup, it becomes very imperative to combine sample collection, extraction, stabilization and storage into a single analytical operation that could accomplish this at the sampling point within several seconds.

This work will seek to design and test a Gas-Sensing technique that will inculcate sampling, extraction and detection of Hydrocarbons (HCs) and possibly other petroleum concomitant gases such as methane (CH_4), Carbon IV oxide (CO_2) and other gases as would be appropriate.
It is hoped that by integrating intrinsic valid measurement into field Portable Gas-sensing Unit for soil analysis will lead to a new advanced methodology for rapid on-site screening of hydrocarbon contaminants from petroleum spilled land sites. This would underscore the possibility of designing an ideal portable

device such a GC to undertake and finalize on-site analysis of these contaminants.

If this research goal is achieved, it will lead to drastic reduction in:

- Labour and cost of sampling
- Sample size and time
- Analyte loss
- Solvent usage and will obviously improve reliability.

6.4 References

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APPENDIX 1

CHAPTER 3

Preparation of Stock Solutions of BTEX

Concentration of Benzene

1000 ppm stock solution of benzene was prepared by measuring 11.5µl of benzene into 10 mL volumetric flask and making up to mark by methanol solvent using micropipette Ependorf.

Standard solutions were from the stock by serial dilution to give the following range of concentrations: 10 – 100ppm.

The calculations were made as detailed below:

Density (sp.gr.) = 0.87 g/cm^3

1000 ppm of benzene was prepared based on mass: density relationship.

Density = Mass/volume and Volume = mass/density

$$V = \frac{m}{d}$$

Assume 1g ≈ 1 mL

$$\approx V = \frac{m}{d} = \frac{0.01g}{0.87g/cm^3} = 0.01149\ cm^3 \approx 0.0115mL \approx 11.5\ \mu l$$

1g ≡1000000 ≡ 10^6 µg ≡ 1000mg

11.5µl benzene/mL ≡10,000 ppm

11.5µl/10 ml ≡10,000µl/10 mL ≡ 1000µl/mL

⇒11.5 µl in 1 mL ≡**1000 ppm**

Preparation of Standard concentrations:

10ppm standard was prepared by diluting the **1000** ppm stock solution using the formula:

$C_1V_1 = C_2V_2$

$$\boxed{V_1 = \frac{C_2V_2}{C_1}} = \frac{10\,ppm \times 10mL}{1000\,ppm} = \frac{1}{10}mL = 0.1 \text{ mL.}$$

0.1mL ≡100µl/10mL

$\Rightarrow$ 01.mL ≡10µl/mL

Therefore, 100µl of Stock solution was taken into 10mL volumetric flask or 10µl was made up to 1.0mL in a GC vial using ependorf micropipette to give 10 ppm standard.

Similarly, 20, 30, 40, 50, 60, and 70 µl etc were measured to prepare 20, 30 to 100ppm.

Standard solutions used for the preparation and plotting of calibration curves for Toluene, Ethylbenzene, and O- xylene were similarly prepared with respect to their densities.

Concentration of n-pentadecane

0.1mL (100µl) of n-pentadecane ($C_{15}H_{32}$) was measured with micropipette (ependorf) into 10mL volumetric flask and made up to mark with heptane. This forms the stock solution whose concentration was calculated as follows:

Mass of $C_{15}H_{32}$ = density x volume

= 0.769gcm-3 x 100ul

= 0.769 gcm^{-3} x 0.1cm^{-3}

= 7.69 x 10^{-2}

Concentration of $C_{15}H_{32}$ in 10ml volume = mass of solute/volume of solution

=7.69 x 10^{-2}g /10cm^{-3}

=7.69 x 10-3gcm^{-3} ≈7.7 x 10^{-3} gcm^{-3} .

On- column mass of the stock

= conc x Inj. Vol. / split ratio

= 7.69 x 10-3gcm^{-3} x 10^{-3} /100

= 76 x10^{-9} g

= 76 ng

Six standard concentrations were prepared from the stock by diluting 100ul, 200ul, 300ul. 400ul, 500ul, 600ul respectively in 10 mL heptane.

Concentration table, number of injections and average peak height from injection counts (signals) for the six standard solutions shown below.

Calibration Parameters of n-pentadecane

Conc. ($g\ cm^{-3}$)	**Peak Area** (mV s) 3 injections			**Average Peak Area** (mV s)	**Retention Time** (s)
	1	2	3		
7.69×10^{-4}	54.3	53.7	48.9	52.3	13.5
15.3×10^{-4}	107	104	107	106.0	13.5
23.1×10^{-4}	152	160	157	156.3	13.5
30.76×10^{-4}	209	191	189	196.3	13.5
38.4×10^{-4}	248	236	280	254.7	13.5
46.1×10^{-4}	288	296	310	298	13.5

APPENDIX II

A

A picture of all the samples on laboratory bench for weighing and extraction

Appendix II

B

Extraction of Petroleum Contaminated soils using DCM and Buchi Extraction Apparatus

Type: Soil **A1** from Petroleum spillage site.

Extraction solvent: Dichloromethane (DCM)

Solvent Extraction Volume: 100 mL

Extraction Time: **2** hours optimum

Weight of Soil: 10 g (average)

NB: Before extraction, about 10g of dried soil A1 was carefully weighed into an extraction thimble and placed in the extraction flask. Actually weights were determined from below.

Sample ID:	**A1.1**	**A1.2**	**A1.3**
Weight of empty	32.2057	32.1975	32.2018
Weight of beaker + Thimble	35.0385	34.8958	34.9125
Wt. beaker + Thimble + Sample	45.1987	44.9003	44.9706
Actual weight of sample	10.1602	10.0046	10.0581

All measurements were made in grammes (g) to four (4) decimal places.

Appendix II

C

Type: Soil **A2** from Petroleum spillage site.

Extraction solvent: Dichloromethane (DCM)

Solvent Extraction Volume: 100 mL

Extraction Time: **2** hours optimum

Weight of Soil: 10 g (average)

NB: Before extraction, about 10g of dried soil A1 was carefully weighed into an extraction thimble and placed in the extraction flask. Actually weights were determined from below.

Sample ID:	**A2.1**	**A2.2**
Weight of empty	32.0906	32.0162
Weight of beaker + Thimble	35.0061	34.0192
Wt. beaker + Thimble + Sample	45.4093	45.2524
Actual weight of sample	10.4032	10.2463

Appendix II

D

Type: Soil **A3** from Petroleum spillage site.

Extraction solvent: Dichloromethane (DCM)

Solvent Extraction Volume: 100 mL

Extraction Time: **2** hours optimum

Weight of Soil: 10 g (average)

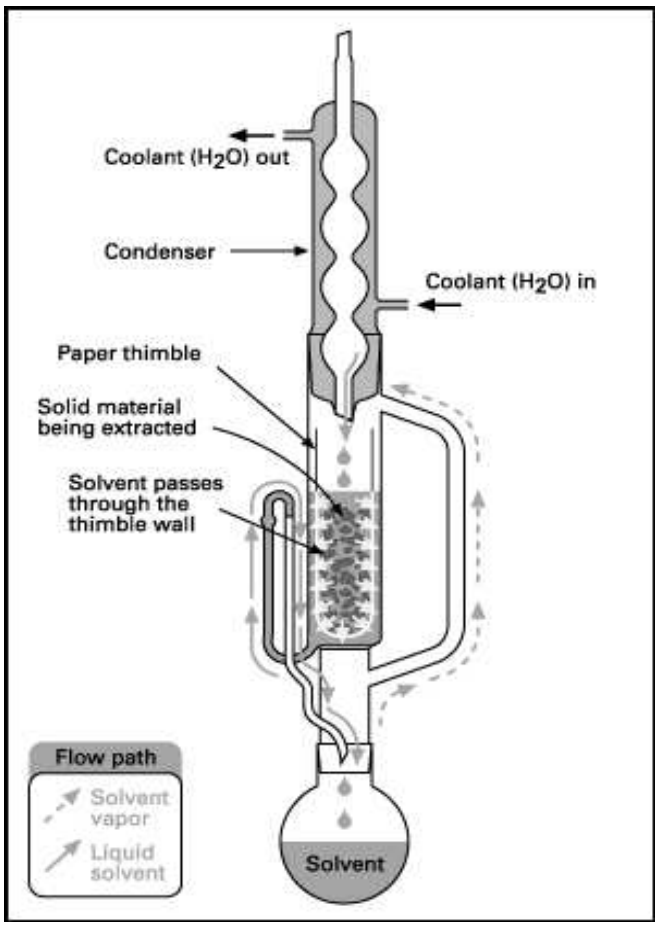
NB: Before extraction, 10g of dried soil A1 was carefully weighed into an extraction flask.

Sample ID:	**A3.1**	**A3.2**	**A3.3**
Weight of empty	32.2107	32.2241	32.2262
Weight of beaker + Thimble	35.1006	35.0296	35.0352
Wt. beaker + Thimble + Sample	45.1033	45.1439	45.0384
Actual weight of sample	10.0027	10.1143	10.0032

NB: The above weighing process for samples A1-A3 was applied to other samples, and recorded.

APPENDIX II
E

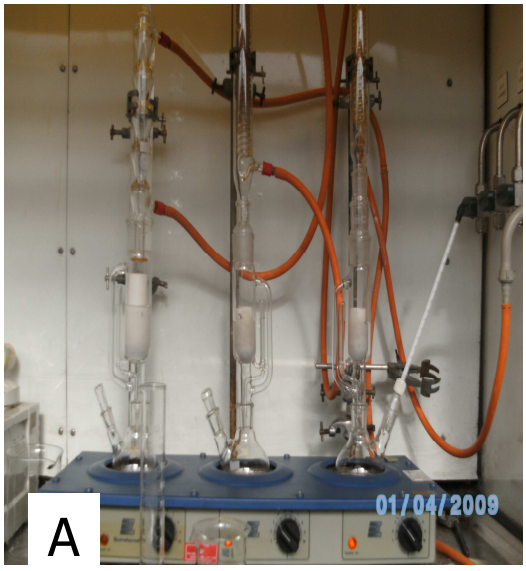
Picture of Soxhlet Apparatus

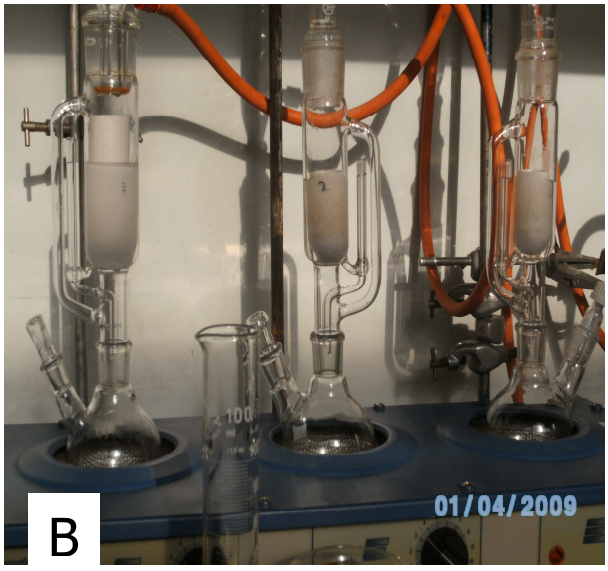


APPENDIX II

F

Picture of Soxhlet Apparatus Set up in the Laboratory Fume Hood. More than 3 samples were extracted at the same time as shown in A-C.

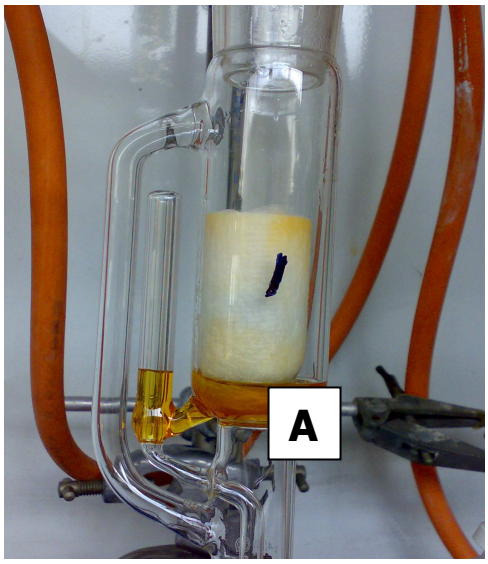


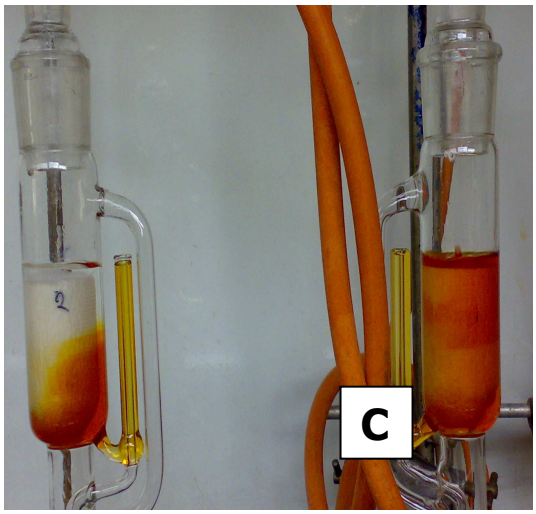


APPENDIX II
G

Soxhlet extraction set-up in Fume Cupboard.

Extraction with DCM. Consistent extraction with DCM (A-D).

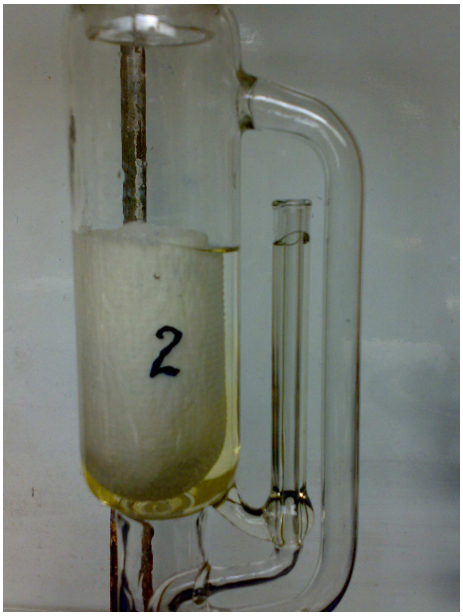


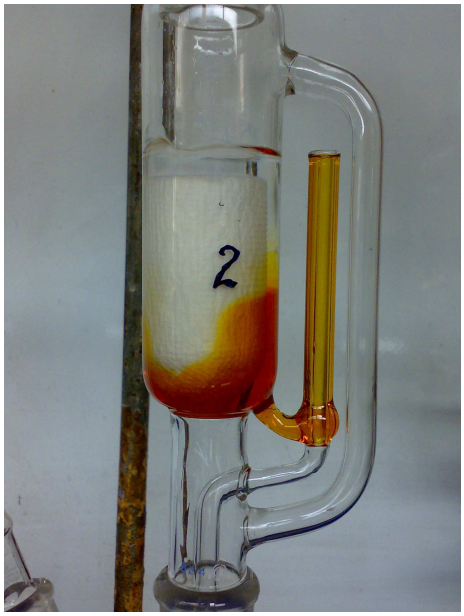


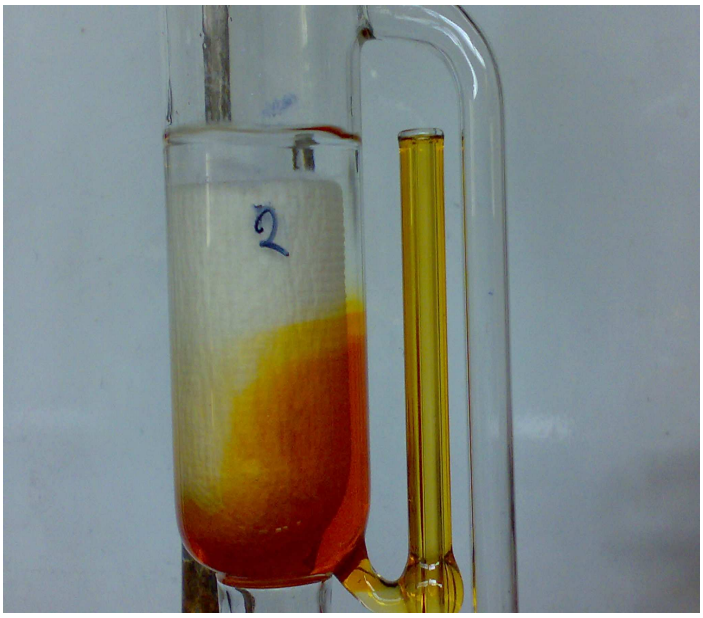
APPENDIX II

H

Extraction using mixture of DCM and Acetone at the same time was not as consistent as with DCM above.







APPENDIX 11 (I)

Chemical Safety Data of DCM

Chemical Safety Data: Dichloromethane

Common synonyms	Methylene chloride, Freon 30
Formula	CH_2Cl_2
Physical properties	Form: colourless liquid Stability: Stable Melting point: -97C Boiling point: 40 C Specific gravity: 1.32
Principal hazards	*** Dichloromethane is harmful if you swallow or inhale it. *** It may act as a narcotic, so inhaling it will make you feel unwell. *** Like many small hydrocarbons that contain halogen atoms, dichloromethane is a suspected carcinogen. It is unlikely to be strongly carcinogenic, but it is important to reduce your exposure to the lowest level possible.
Safe handling	Wear safety glasses. Work in a well ventilated area. Avoid repeated or long-lasting exposure.
Emergency	Eye contact: Immediately flush the eye with water. If irritation persists, call for medical help. Skin contact: Wash off with soap and water. If swallowed: Call for medical help.
Disposal	Store for later disposal as chlorinated waste solvent.
Protective equipment	Safety glasses.

Source: H-Sci Project: Chemical safety database. Oxford.

APPENDIX II

J

A Typical Sample Extract in a flask after complete extraction

APPENDIX 111

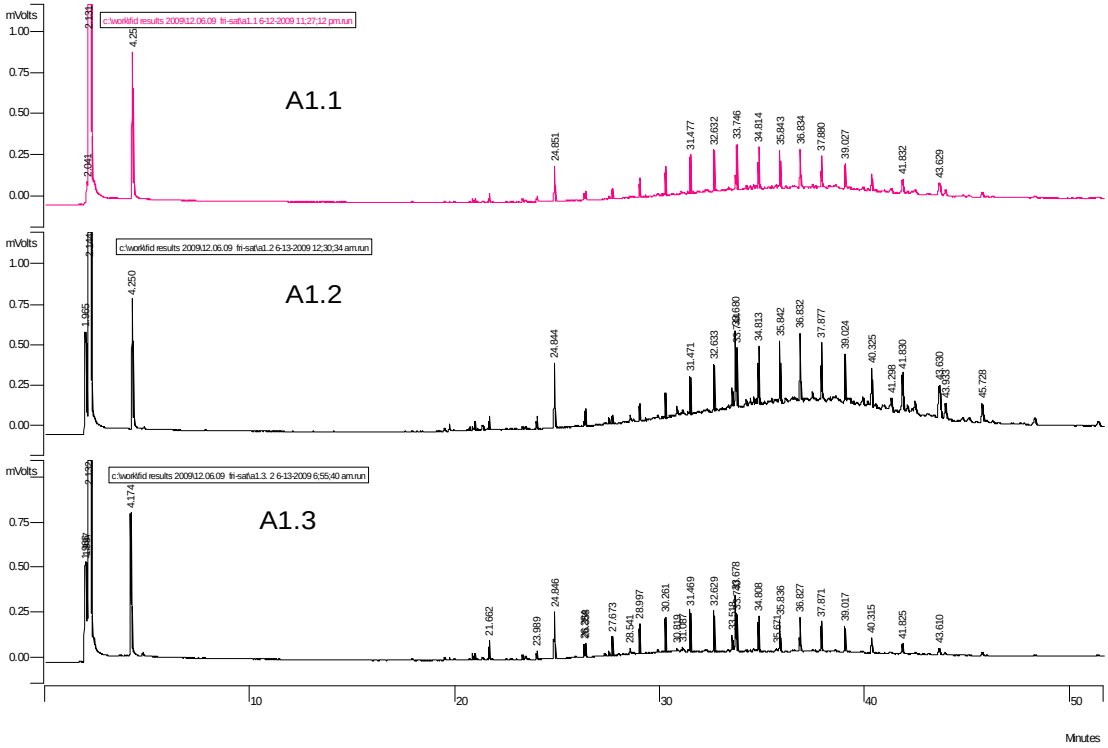
DATA STATISTICS OF THE ENTIRE SAMPLES

Soil		Average. HC Peak Area for Soil Levels						
		Average	**1**	**2**	**3**	**STDEV**	**% RSD**	**95%**
1	A1.1	**545.56**	548.57	551.83	536.27	8.206006	1.504153	24.968
	A1.2	**889.32**	898.77	886.93	882.27	8.506382	0.9565	25.88
	A1.3	**438.75**	430.42	432.22	453.62	12.90633	2.941591	39.269
2	A2.1	**796.59**	798.37	808.24	783.16	12.63439	1.58606	38.442
	A2.2	**388.28**	389.79	381.29	393.75	6.36636	1.639645	19.37
3	A3.1	**1343.09**	1342.7	1344.85	1341.73	1.596757	0.118887	4.8584
	A3.2	**1697.48**	1697.44	1702.68	1692.33	5.175136	0.304871	15.746
	A3.3	**933.00**	931.02	938.43	929.55	4.759611	0.510141	14.481
4	A4.1	**442.47**	425.67	449.87	451.86	14.58033	3.295238	44.363
	A4.2	**482.40**	489.22	479.99	477.98	5.994033	1.242553	18.237
	A4.3	**482.34**	487.67	479.23	480.12	4.637316	0.961421	14.109
5	A5.1	**604.70**	603.33	608.83	601.95	3.639799	0.601915	11.074
	A5.2	**514.91**	513.6	516.48	514.66	1.456617	0.282886	4.432
	A5.3	**559.85**	561.00	558.88	559.66	1.072256	0.191527	3.2625
6	A6.1	**54.02**	51.5	55.98	54.57	2.290684	4.240699	6.969
	A6.2	**47.85**	47.56	47.86	48.13	0.285132	0.595886	0.8675
7	A7.1	**45.26**	45.00	45.9	44.87	0.560922	1.239424	1.7067
	A7.2	**51.33**	51.55	50.8	51.65	0.464579	0.905023	1.4135
	A7.3	**45.90**	45.75	46.68	45.27	0.716868	1.561804	2.1812
8	A8.1	**258.47**	254.56	259.45	261.4	3.523734	1.363305	10.72
	A8.2	**322.91**	325.42	320.76	322.55	2.350766	0.727994	7.1526
	A8.3	**344.99**	349.75	343.51	341.72	4.215499	1.221907	12.826
9	A9.1	**248.14**	241.67	249.21	253.53	6.002411	2.418994	18.263
10	A10.1	**269.23**	265.55	271.66	270.47	3.239203	1.203151	9.8558
11	A11.1	**1667.77**	1662.84	1672.61	1667.85	4.885533	0.292939	14.865
	A11.2	**1819.77**	1818.61	1821.12	1819.59	1.265003	0.069514	3.8490
12	A12.1	**282.94**	286.67	278.48	283.66	4.142636	1.464157	12.604

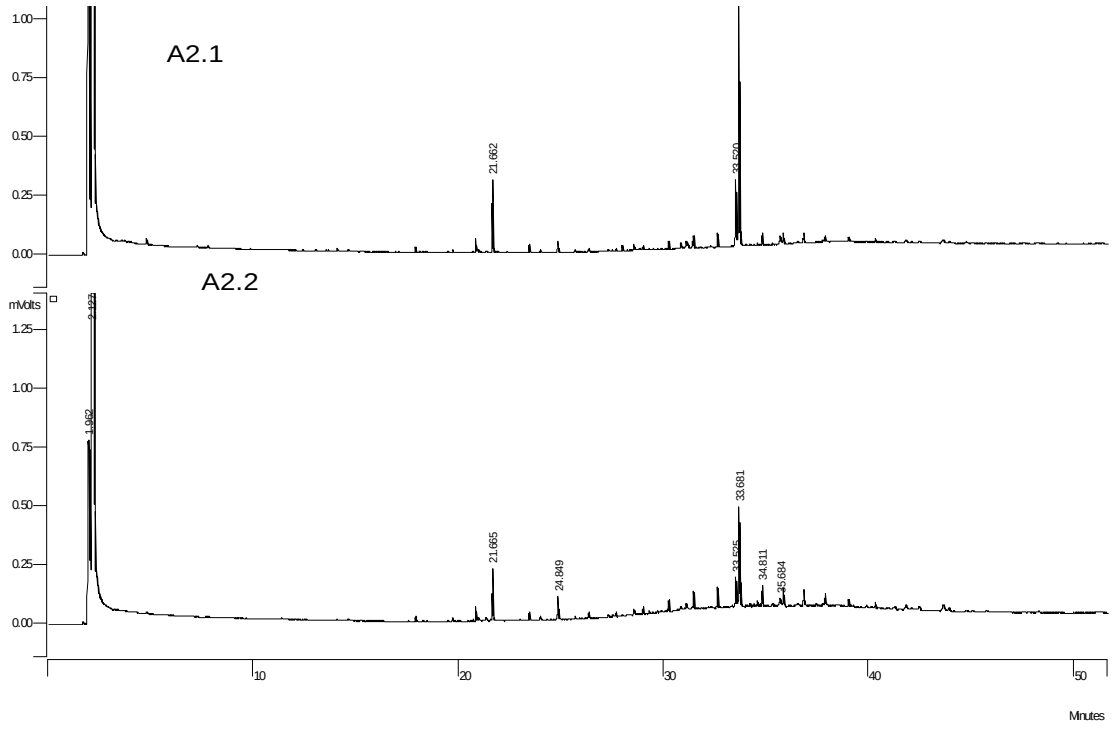
APPENDIX IV

Chromatograms of all the samples displaying the three depths of analysis with the Retention times as applied in figures 5.13-5.24.

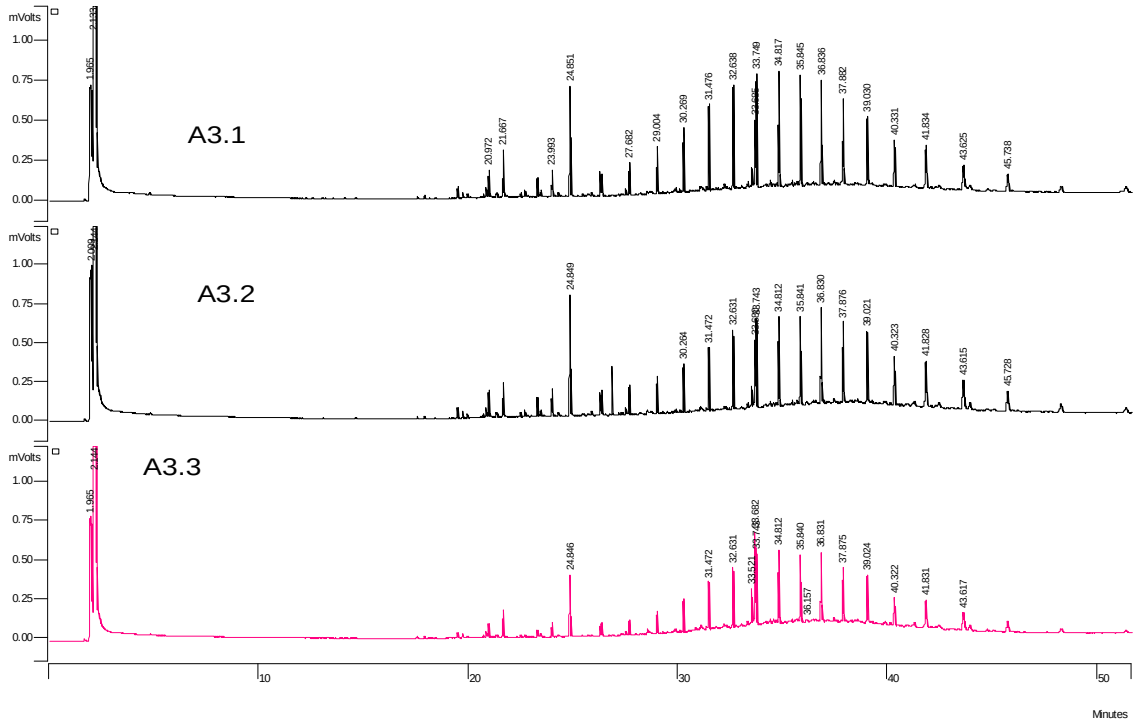
Soil Sample A1 (top soil A1.1, middle soil A1.2, bottom soil A1.3)



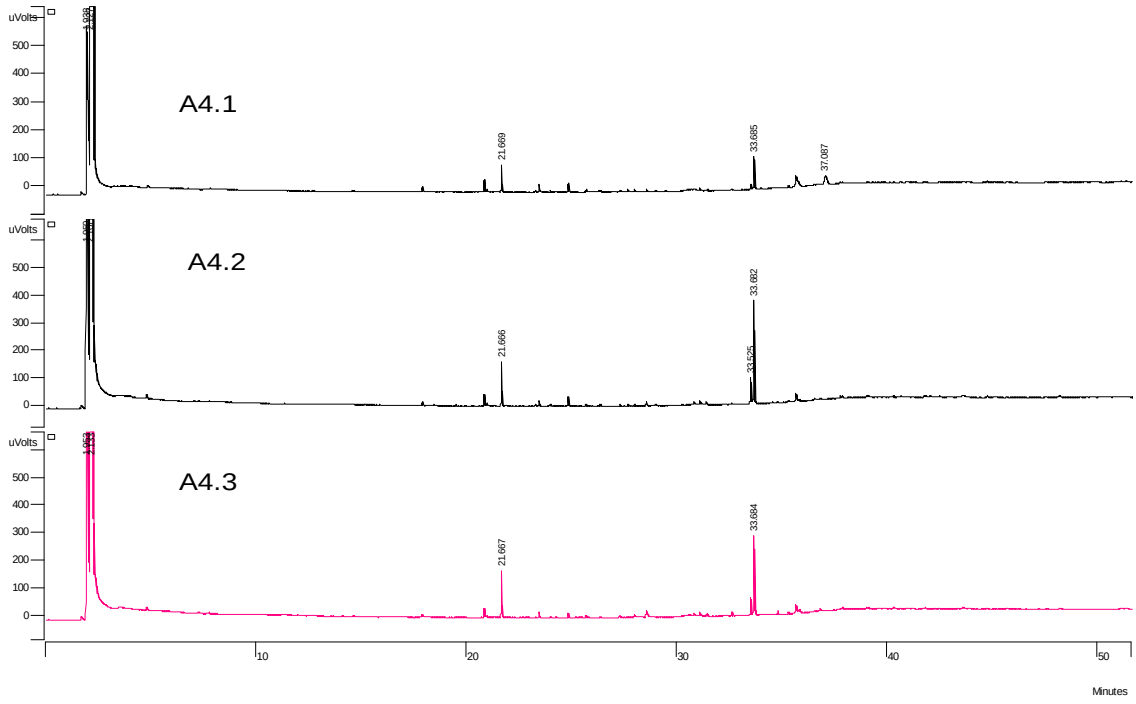
Soil Sample A2 (top soil A2.1, middle soil A2.2,



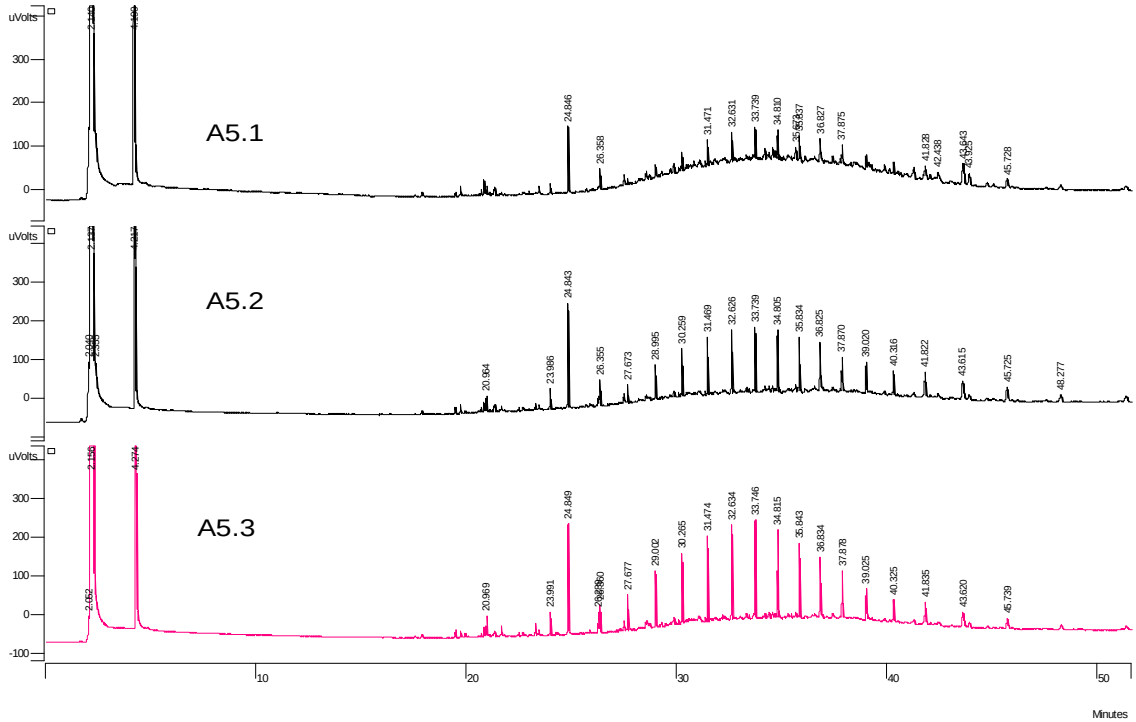
Soil Sample A3 (top soil A3.1, middle soil A3.1, bottom soil A3.3)



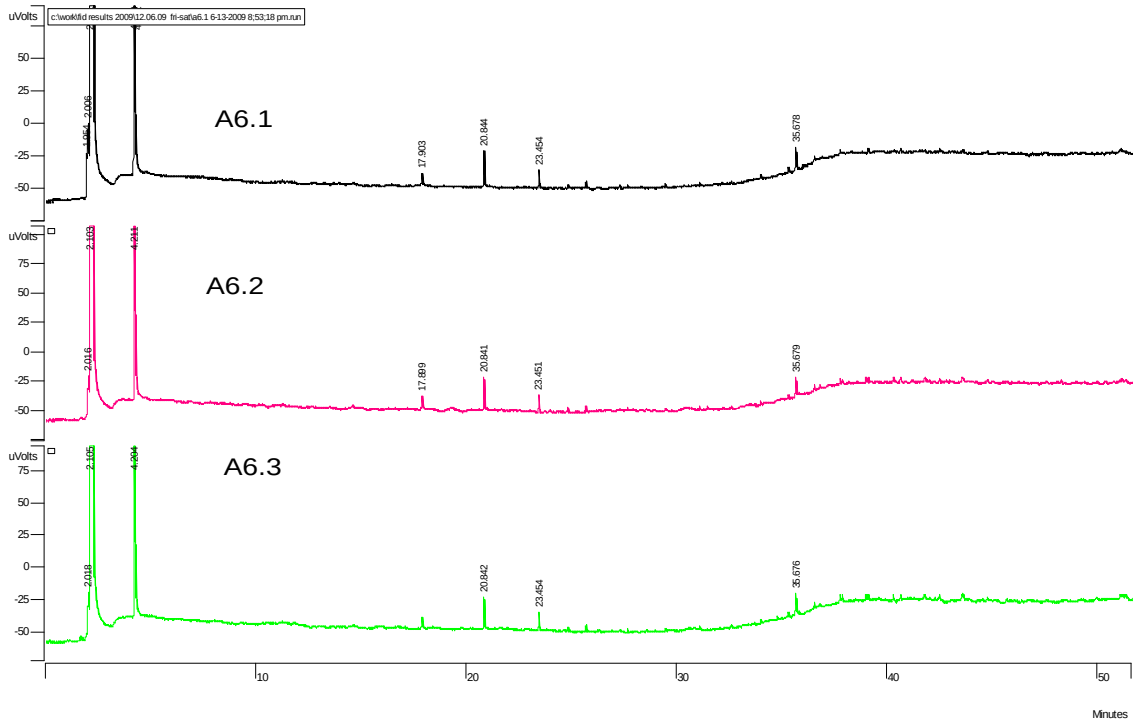
Soil Sample A4 (top soil A4.1, middle soil A4.1, bottom soil A4.3)



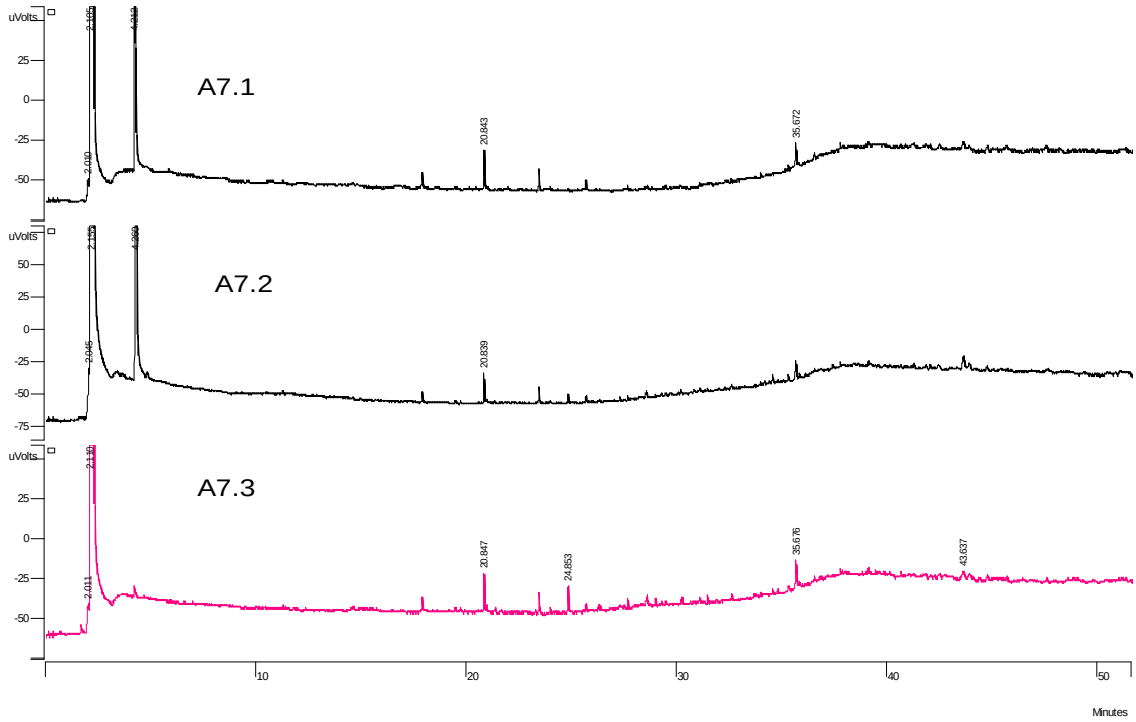
Soil Sample A5 (top soil A5.1, middle soil A5.1, bottom soil A5.3)



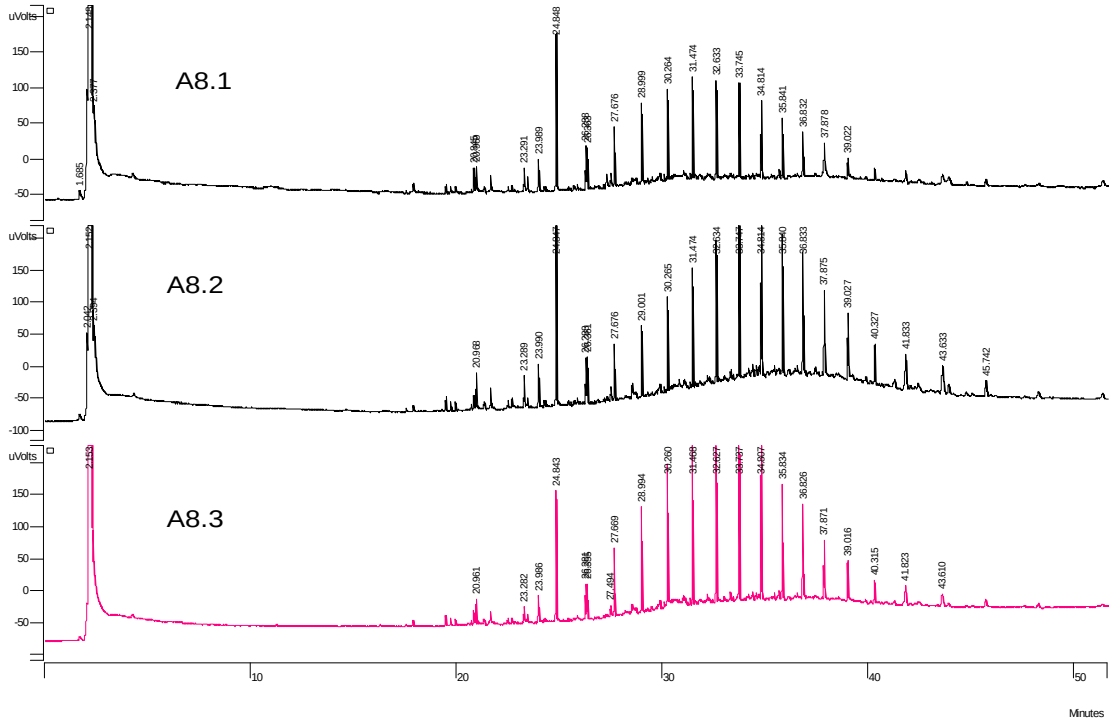
Soil Sample A6 (top soil A6.1, middle soil A6.1, bottom soil A6.3)



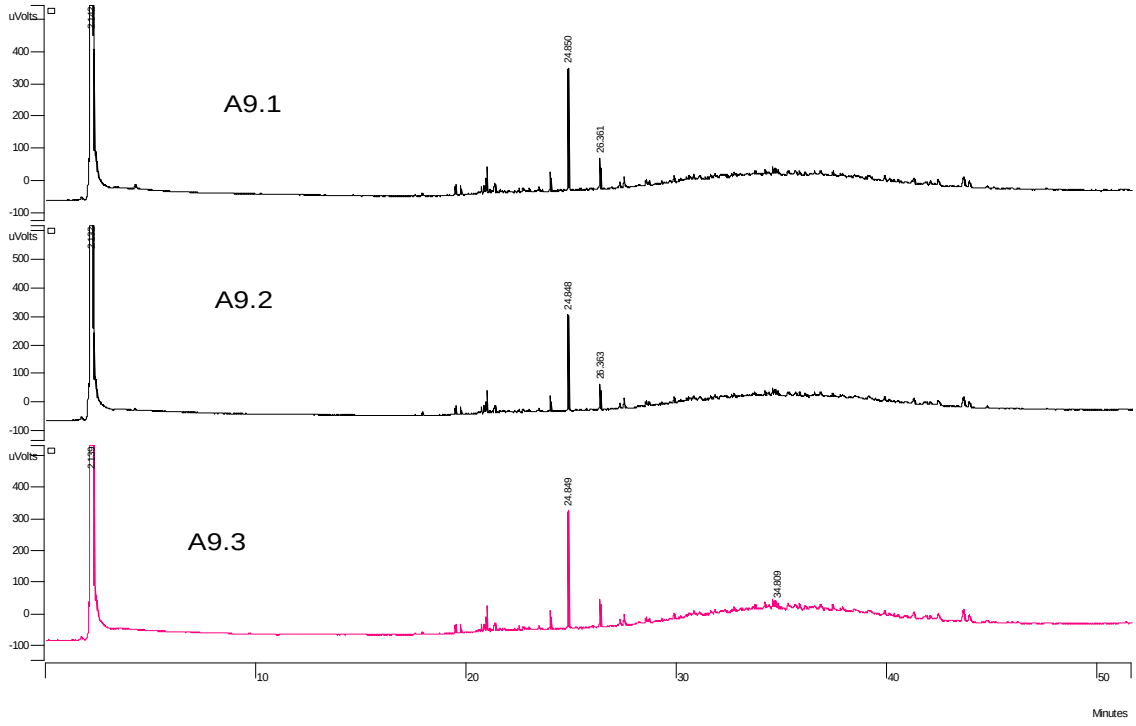
Soil Sample A7 (top soil A7.1, middle soil A7.1, bottom soil A7.3)



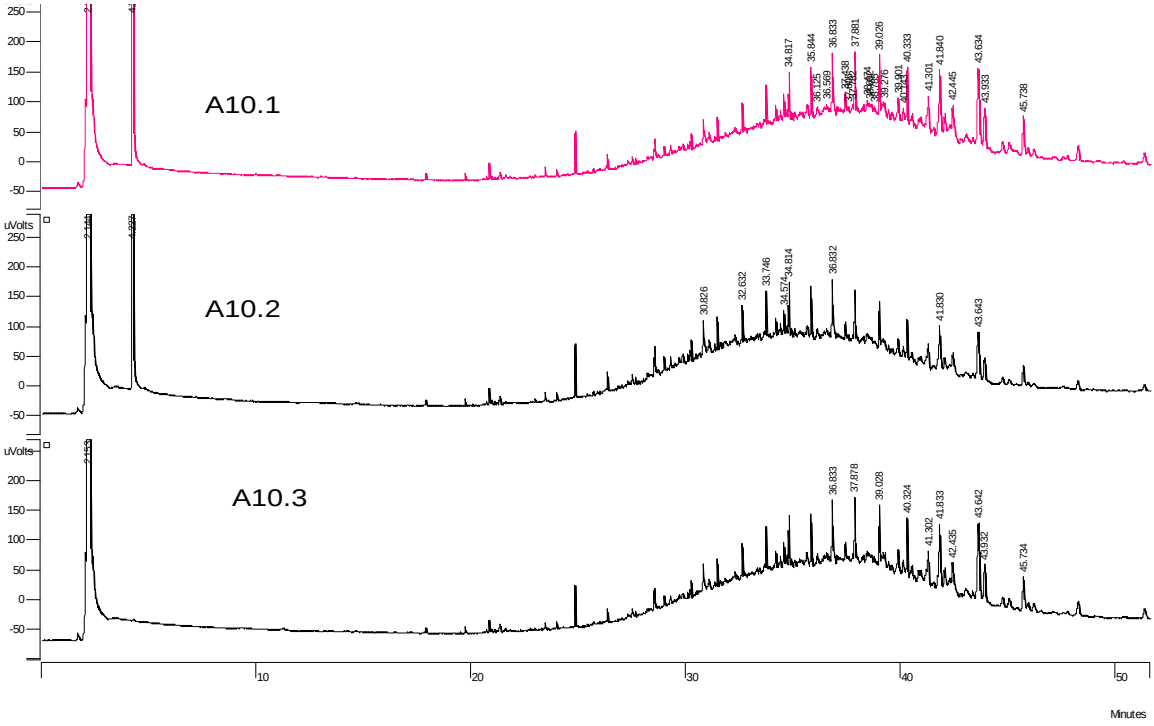
Soil Sample A8 (top soil A8.1, middle soil A8.1, bottom soil A8.3)



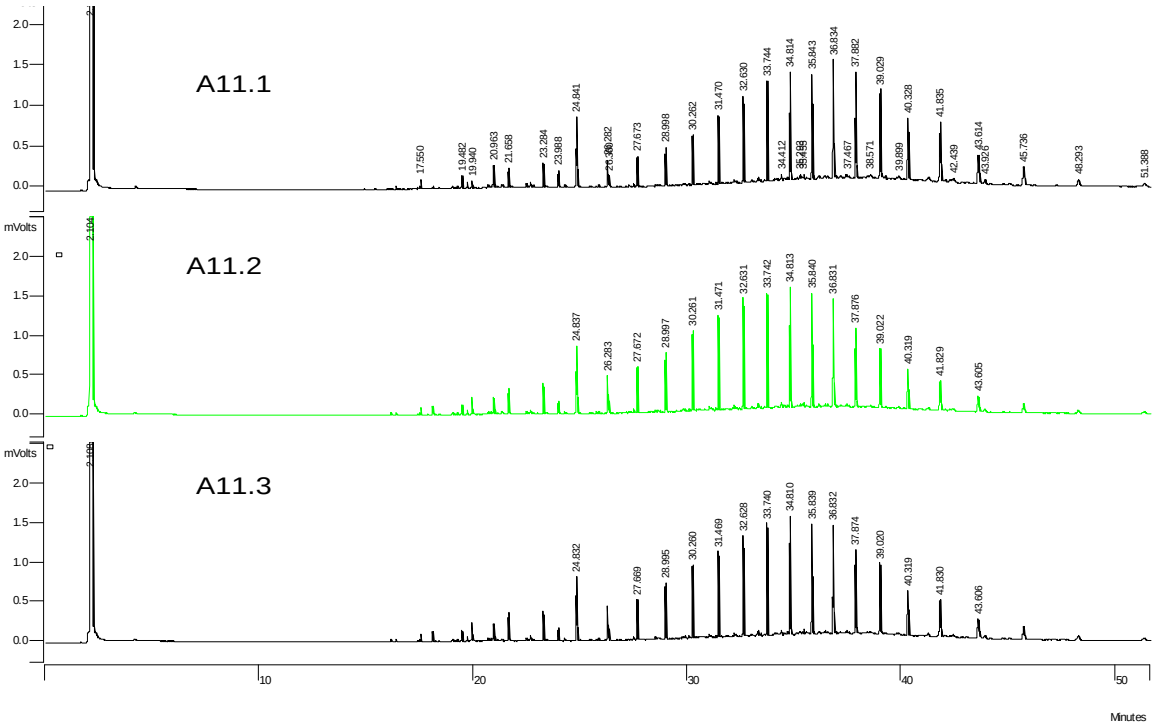
Soil Sample A9 (top soil A9.1, middle soil A9.1, bottom soil A9.3)



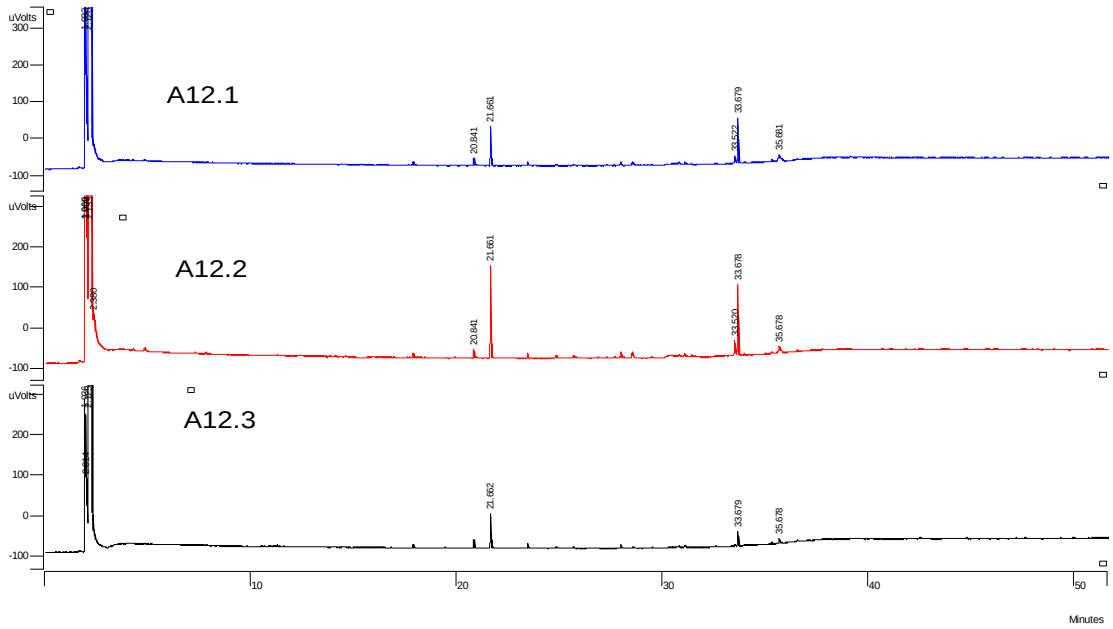
Soil Sample A10 (top soil A10.1, middle soil A10.1, bottom soil A10.3)



Soil Sample A11 (top soil A11.1, middle soil A11.1, bottom soil A11.3



Soil Sample A12 (top soil A12.1, middle soil A12.1, bottom soil A12.3)



APPENDIX V
A

Principal Component Analysis: The Raw Data

Eigenanalysis of the Covariance Matrix

Eigenvalue	0.096472	0.035786	0.008041	0.005209	0.002015	0.001220
Proportion	0.638	0.237	0.053	0.034	0.013	0.008
Cumulative	0.638	0.874	0.927	0.962	0.975	0.983
Eigenvalue	0.000883	0.000493	0.000362	0.000277	0.000194	0.000107
Proportion	0.006	0.003	0.002	0.002	0.001	0.001
Cumulative	0.989	0.992	0.995	0.996	0.998	0.998
Eigenvalue	0.000080	0.000067	0.000034	0.000026	0.000014	0.000006
Proportion	0.001	0.000	0.000	0.000	0.000	0.000
Cumulative	0.999	0.999	1.000	1.000	1.000	1.000
Eigenvalue	0.000004	0.000002	0.000000	0.000000	0.000000	0.000000
Proportion	0.000	0.000	0.000	0.000	0.000	0.000
Cumulative	1.000	1.000	1.000	1.000	1.000	1.000

Variable	PC1	PC2	PC3	PC4	PC5	PC6
C1	0.984	0.104	0.136	0.043	-0.009	0.022
C2	-0.017	-0.121	0.079	0.403	-0.218	-0.584
C3	0.086	-0.972	0.150	-0.110	-0.007	0.099
C4	0.019	-0.032	0.104	-0.046	0.077	-0.714
C5	-0.043	-0.040	0.128	0.732	0.103	0.182
C6	-0.046	-0.003	0.164	0.489	-0.085	0.206
C7	-0.037	0.034	0.195	-0.031	0.200	0.005
C8	-0.036	0.034	0.255	-0.060	0.252	0.103
C9	-0.042	0.042	0.310	-0.065	0.222	0.007
C10	-0.046	0.049	0.339	-0.068	0.208	0.015
C11	-0.051	0.054	0.359	-0.064	0.166	-0.032
C12	-0.044	0.053	0.300	-0.059	-0.029	-0.007
C13	-0.053	0.061	0.362	-0.046	0.038	-0.078
C14	-0.046	0.052	0.295	-0.068	-0.135	0.030
C15	-0.041	0.042	0.236	-0.062	-0.194	0.085
C16	-0.037	0.043	0.206	-0.058	-0.269	0.094
C17	-0.021	0.036	0.130	-0.030	-0.235	0.007
C18	-0.013	0.038	0.126	-0.050	-0.365	-0.094
C19	-0.016	0.026	0.100	-0.064	-0.379	0.045
C20	-0.010	0.010	0.038	-0.023	-0.192	0.053
C21	-0.015	0.018	0.064	-0.054	-0.383	0.106
C22	-0.008	0.009	0.028	-0.024	-0.216	0.056
C23	-0.004	0.005	0.011	-0.009	-0.108	0.028
C24	-0.001	0.002	0.004	-0.004	-0.040	0.010

Variable	PC7	PC8	PC9	PC10	PC11	PC12
C1	-0.018	0.001	-0.000	-0.000	-0.009	-0.005
C2	-0.619	-0.075	0.062	0.117	-0.105	0.002
C3	0.035	0.011	-0.023	-0.004	0.018	-0.011
C4	0.631	-0.070	0.045	-0.184	-0.140	0.079
C5	0.244	0.105	0.112	-0.026	0.208	0.239
C6	0.206	-0.146	-0.140	-0.057	-0.229	-0.337
C7	-0.080	-0.097	-0.070	-0.104	-0.354	-0.622
C8	-0.055	-0.352	0.209	-0.002	-0.252	0.348
C9	-0.020	-0.128	0.251	0.125	0.073	-0.163
C10	-0.022	-0.229	0.097	0.370	0.076	0.140
C11	-0.031	0.158	0.052	0.168	0.120	-0.077
C12	-0.144	-0.259	-0.192	-0.668	0.475	-0.054
C13	-0.008	0.744	-0.142	0.013	0.013	-0.025
C14	-0.083	0.063	-0.060	-0.020	-0.006	0.090
C15	0.014	0.142	-0.013	-0.065	-0.229	0.106
C16	-0.071	0.003	-0.113	-0.173	-0.303	0.400

C17	0.114	-0.258	-0.627	0.250	-0.049	0.112
C18	0.116	-0.105	0.032	0.231	0.466	-0.182
C19	0.136	-0.062	0.358	0.176	0.110	-0.086
C20	0.137	-0.048	-0.102	0.254	-0.101	-0.096
C21	0.031	0.019	0.471	-0.244	-0.165	-0.041
C22	0.056	0.006	0.092	-0.064	-0.115	-0.112
C23	0.055	-0.014	-0.036	0.036	-0.074	-0.058
C24	0.016	-0.009	0.024	0.014	-0.024	-0.019

Variable	PC13	PC14	PC15	PC16	PC17	PC18
C1	0.003	0.000	-0.001	-0.003	-0.002	-0.003
C2	0.038	0.079	-0.038	-0.029	0.035	-0.005
C3	-0.010	-0.007	0.012	0.003	-0.001	0.001
C4	0.021	-0.053	-0.026	0.011	-0.032	0.013
C5	0.048	-0.014	0.130	-0.102	-0.205	-0.350
C6	-0.102	-0.045	-0.159	0.163	0.258	0.499
C7	-0.062	-0.193	0.375	-0.219	-0.170	-0.331
C8	-0.320	0.417	0.183	-0.045	0.244	-0.119
C9	-0.171	0.112	-0.435	0.235	-0.041	-0.054
C10	0.551	-0.373	0.224	0.081	0.205	0.152
C11	-0.074	0.164	-0.140	-0.124	-0.438	0.307
C12	0.213	0.112	-0.049	-0.174	0.087	0.035
C13	0.011	0.243	0.252	0.026	0.288	0.068
C14	-0.015	-0.270	-0.169	0.358	-0.401	-0.218
C15	-0.008	-0.205	-0.445	-0.189	0.371	-0.395
C16	-0.216	-0.345	0.118	-0.155	-0.167	0.299
C17	0.012	0.331	0.152	0.127	-0.174	-0.112
C18	-0.450	-0.200	0.209	0.161	0.252	-0.152
C19	-0.027	0.097	0.081	-0.535	-0.112	0.146
C20	0.312	0.156	-0.277	-0.331	0.005	-0.126
C21	0.221	0.132	0.227	0.333	-0.066	-0.004
C22	0.142	0.273	-0.055	0.205	-0.138	-0.055
C23	0.233	0.114	0.008	0.138	0.138	-0.078
C24	0.153	0.072	0.109	0.083	0.016	-0.057

Variable	PC19	PC20	PC21	PC22	PC23	PC24
C1	-0.001	0.000	-0.000	-0.000	-0.000	0.000
C2	-0.009	-0.002	-0.001	-0.000	0.000	0.000
C3	-0.000	0.000	0.000	0.000	0.000	-0.000
C4	0.026	-0.003	0.000	-0.003	-0.000	-0.000
C5	-0.134	0.042	-0.048	-0.011	-0.000	0.000
C6	0.201	-0.060	0.069	0.016	0.000	-0.000
C7	-0.070	0.009	-0.033	-0.011	-0.000	0.000
C8	0.290	0.019	-0.010	-0.150	0.062	0.061
C9	-0.414	0.119	-0.101	0.104	-0.348	-0.318
C10	-0.047	-0.092	-0.031	0.033	0.151	-0.082
C11	-0.063	0.055	0.182	-0.025	0.284	0.530
C12	0.018	0.022	-0.012	-0.031	-0.014	-0.026
C13	0.041	-0.023	-0.048	-0.046	-0.117	-0.216
C14	0.609	-0.115	-0.054	-0.077	-0.158	-0.073
C15	-0.107	-0.155	0.162	0.277	0.219	0.218
C16	-0.297	0.327	-0.181	-0.094	-0.065	-0.092
C17	-0.223	-0.238	0.151	0.214	-0.085	-0.037
C18	-0.037	0.184	0.009	-0.192	0.162	0.107
C19	0.208	-0.224	-0.166	0.343	-0.207	-0.142
C20	0.075	0.296	0.141	-0.615	-0.071	-0.145
C21	-0.233	-0.168	0.387	-0.176	-0.113	0.117
C22	-0.023	0.119	-0.443	0.085	0.676	-0.266
C23	-0.022	0.142	-0.597	0.006	-0.352	0.600
C24	0.216	0.729	0.331	0.502	-0.071	0.000

APPENDIX V
B

Data Information for Cluster Analysis

Number of clusters: 24

	Number of observations	Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
Cluster1	1	0.000	0.000	0.000
Cluster2	1	0.000	0.000	0.000
Cluster3	1	0.000	0.000	0.000
Cluster4	1	0.000	0.000	0.000
Cluster5	1	0.000	0.000	0.000
Cluster6	1	0.000	0.000	0.000
Cluster7	1	0.000	0.000	0.000
Cluster8	1	0.000	0.000	0.000
Cluster9	1	0.000	0.000	0.000
Cluster10	3	0.527	0.401	0.575
Cluster11	1	0.000	0.000	0.000
Cluster12	1	0.000	0.000	0.000
Cluster13	1	0.000	0.000	0.000
Cluster14	1	0.000	0.000	0.000
Cluster15	1	0.000	0.000	0.000
Cluster16	1	0.000	0.000	0.000
Cluster17	1	0.000	0.000	0.000
Cluster18	6	0.000	0.000	0.000
Cluster19	1	0.000	0.000	0.000
Cluster20	2	0.542	0.520	0.520
Cluster21	2	0.062	0.176	0.176
Cluster22	1	0.000	0.000	0.000
Cluster23	1	0.000	0.000	0.000
Cluster24	1	0.000	0.000	0.000

Cluster Centroids

Variable	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
C1	-0.1002	-0.6991	-0.0749	-0.6219	-0.5950
C2	-0.0503	0.2292	-0.1959	0.8662	0.8360
C3	-0.3274	-0.2544	-0.2134	-0.4940	-0.5999
C4	-0.2503	-0.6630	-0.0997	0.2127	-0.6630
C5	-0.3868	-0.3868	0.0135	0.3411	0.1588
C6	-0.3888	-0.0536	0.1288	0.7184	0.4178
C7	-0.2594	0.6542	0.6387	1.8746	1.3127
C8	0.1082	1.0342	0.9654	0.6824	0.8499
C9	0.1127	1.1666	0.8418	1.3045	1.0112
C10	0.7644	-0.0448	1.3053	1.4502	1.2498
C11	0.2041	1.0809	0.5287	1.4281	1.3117
C12	0.5275	1.5112	0.7297	1.9239	1.9934
C13	0.3065	1.1112	0.4262	1.1098	1.5305
C14	0.9013	1.5774	0.8605	1.3455	2.0364
C15	0.8802	1.7533	1.1538	0.7151	1.5166
C16	1.0378	1.9780	1.2377	1.0454	1.6735
C17	0.2215	-0.5932	-0.5932	0.9707	1.2931
C18	1.1093	1.5781	0.8283	0.3041	0.5777
C19	2.3894	2.2255	0.7547	-0.5630	-0.5630
C20	1.4564	-0.4044	-0.4044	-0.4044	-0.4044
C21	3.2371	2.8833	0.8553	-0.3922	-0.3922
C22	2.6071	2.1333	-0.3613	-0.3613	-0.3613
C23	2.4639	-0.3054	-0.3054	-0.3054	-0.3054
C24	3.7310	-0.2500	-0.2500	-0.2500	-0.2500

Variable	Cluster6	Cluster7	Cluster8	Cluster9	Cluster10
C1	-0.6933	1.1275	0.5543	0.5280	2.2326
C2	-0.6173	0.4583	-0.4309	-0.3643	-0.3749
C3	-0.5999	-0.5999	-0.5266	-0.5028	-0.2912
C4	-0.6630	1.2822	1.3964	2.0197	0.2225
C5	0.3153	-0.3868	-0.0721	0.0272	-0.3868
C6	0.7250	-0.6458	-0.2291	0.2212	-0.6458
C7	2.1073	-0.8587	0.1242	0.8166	-0.8587
C8	3.2204	-0.6737	0.4648	0.8970	-0.6737
C9	1.7301	-0.0736	0.5096	0.7583	-0.7783
C10	1.8910	-0.0419	0.4427	0.5186	-0.7852
C11	1.6219	0.1071	0.4679	0.4548	-0.8104
C12	1.9209	0.0948	0.4430	0.3880	-0.7662
C13	1.1291	0.2061	0.2827	0.1542	-0.7667
C14	1.3156	-0.0282	0.1962	-0.0349	-0.8278
C15	1.0236	-0.7828	0.3491	-0.0445	-0.7828
C16	1.5899	-0.7126	0.2030	-0.1549	-0.7126
C17	1.8105	0.7525	0.7944	0.2361	-0.5932
C18	-0.6577	2.4028	0.9126	0.2724	-0.6577
C19	-0.5630	0.6690	0.4310	-0.0643	-0.5630
C20	-0.4044	-0.4044	0.5146	-0.4044	-0.4044
C21	-0.3922	-0.3922	-0.3922	-0.3922	-0.3922
C22	-0.3613	-0.3613	-0.3613	-0.3613	-0.3613
C23	-0.3054	-0.3054	-0.3054	-0.3054	-0.3054
C24	-0.2500	-0.2500	-0.2500	-0.2500	-0.2500

Variable	Cluster11	Cluster12	Cluster13	Cluster14	Cluster15
C1	2.4296	-0.6991	-0.6991	-0.6991	-0.6439
C2	-0.6592	3.2844	-0.6592	-0.1295	-0.2321
C3	-0.4834	0.7865	0.3233	0.0939	-0.0635
C4	-0.6630	-0.6630	0.4554	0.0522	-0.0372
C5	-0.3868	5.2587	0.3814	0.1605	0.0595
C6	-0.1897	4.6895	0.5698	0.0895	0.2299
C7	-0.8587	-0.8587	1.9323	0.8353	1.4094
C8	-0.6737	-0.6737	2.0655	1.0102	1.7842
C9	-0.7783	-0.7783	2.4280	1.2249	1.9831
C10	-0.7852	-0.7852	1.8736	1.5638	2.0326
C11	-0.8104	-0.8104	1.6005	1.4443	1.8113
C12	-0.7662	-0.7662	1.5560	1.4804	-0.7662
C13	-0.7667	-0.7667	0.7073	0.8047	1.1621
C14	-0.8278	-0.8278	0.7386	0.9632	1.2742
C15	-0.7828	-0.7828	0.4861	0.8309	1.0460
C16	-0.7126	-0.7126	-0.7126	0.5511	0.6774
C17	-0.5932	-0.5932	-0.5932	0.7401	0.5346
C18	-0.6577	-0.6577	-0.6577	0.8136	0.4316
C19	-0.5630	-0.5630	-0.5630	1.2619	0.6636
C20	-0.4044	-0.4044	-0.4044	1.2873	1.0004
C21	-0.3922	-0.3922	-0.3922	-0.3922	-0.3922
C22	-0.3613	-0.3613	-0.3613	-0.3613	-0.3613
C23	-0.3054	-0.3054	-0.3054	-0.3054	-0.3054
C24	-0.2500	-0.2500	-0.2500	-0.2500	-0.2500

Variable	Cluster16	Cluster17	Cluster18	Cluster19	Cluster20
C1	-0.6991	-0.6595	-0.6991	-0.1002	-0.3870
C2	-0.3065	-0.3945	-0.6592	-0.0503	0.0166
C3	-0.5310	-0.5095	-0.5999	-0.3274	-0.2339
C4	-0.1730	-0.0897	-0.6630	-0.2503	-0.3813
C5	-0.1280	-0.1939	-0.3868	-0.3868	-0.1866
C6	1.0557	0.8222	-0.6458	-0.3888	0.0376
C7	-0.0623	0.0529	-0.8587	-0.2594	0.6464
C8	-0.2743	-0.3494	-0.6737	-0.6737	-0.6737
C9	-0.0969	0.0518	-0.7783	-0.7783	-0.7783
C10	0.0332	0.2205	-0.7852	-0.7852	-0.7852
C11	0.1732	0.4241	-0.8104	-0.8104	-0.8104
C12	0.6327	0.8980	-0.7662	-0.7662	-0.7662
C13	0.6197	0.9010	-0.7667	-0.7667	-0.7667
C14	1.1900	1.4966	-0.8278	-0.8278	-0.8278
C15	1.7413	2.0902	-0.7828	-0.7828	-0.7828
C16	1.8203	2.1331	-0.7126	-0.7126	-0.7126
C17	2.8372	2.8601	-0.5932	-0.5932	-0.5932

C18	2.4622	2.1193	-0.6577	-0.6577	-0.6577
C19	2.5758	2.0427	-0.5630	-0.5630	-0.5630
C20	3.7575	2.9021	-0.4044	-0.4044	-0.4044
C21	2.2726	1.7334	-0.3922	-0.3922	-0.3922
C22	3.3235	2.4128	-0.3613	-0.3613	-0.3613
C23	3.9430	2.7541	-0.3054	-0.3054	-0.3054
C24	4.0178	-0.2500	-0.2500	-0.2500	-0.2500

Variable	Cluster21	Cluster22	Cluster23	Cluster24	Grand centrd
C1	0.6610	0.1510	-0.3143	-0.5432	0.0000
C2	-0.6592	3.3123	1.4569	0.0120	0.0000
C3	2.5261	2.3413	2.7170	-0.3404	-0.0000
C4	-0.6630	-0.6630	4.0591	0.7995	0.0000
C5	-0.3868	-0.3868	-0.3868	1.0134	0.0000
C6	-0.6458	-0.6458	-0.6458	0.5473	-0.0000
C7	-0.8587	-0.8587	-0.8587	1.2692	-0.0000
C8	-0.6737	-0.6737	-0.6737	0.3415	0.0000
C9	-0.7783	-0.7783	-0.7783	1.0570	-0.0000
C10	-0.7852	-0.7852	-0.7852	0.8753	0.0000
C11	-0.8104	-0.8104	-0.8104	1.9293	-0.0000
C12	-0.7662	-0.7662	-0.7662	0.4587	0.0000
C13	-0.7667	-0.7667	-0.7667	3.3495	0.0000
C14	-0.8278	-0.8278	-0.8278	1.0688	0.0000
C15	-0.7828	-0.7828	-0.7828	1.3315	-0.0000
C16	-0.7126	-0.7126	-0.7126	0.4589	0.0000
C17	-0.5932	-0.5932	-0.5932	-0.5932	0.0000
C18	-0.6577	-0.6577	-0.6577	-0.6577	-0.0000
C19	-0.5630	-0.5630	-0.5630	-0.5630	0.0000
C20	-0.4044	-0.4044	-0.4044	-0.4044	0.0000
C21	-0.3922	-0.3922	-0.3922	-0.3922	0.0000
C22	-0.3613	-0.3613	-0.3613	-0.3613	-0.0000
C23	-0.3054	-0.3054	-0.3054	-0.3054	-0.0000
C24	-0.2500	-0.2500	-0.2500	-0.2500	-0.0000

Distances Between Cluster Centroids

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
Cluster1	0.0000	6.0753	6.9286	8.5493	8.5070
Cluster2	6.0753	0.0000	4.3455	6.1089	5.7701
Cluster3	6.9286	4.3455	0.0000	3.6168	3.8018
Cluster4	8.5493	6.1089	3.6168	0.0000	1.8060
Cluster5	8.5070	5.7701	3.8018	1.8060	0.0000
Cluster6	9.5013	7.0433	4.9580	3.4521	3.4993
Cluster7	8.1662	7.5016	5.2059	6.2695	6.7378
Cluster8	7.4917	6.3518	3.3162	4.3003	4.8873
Cluster9	8.2109	6.8870	3.6380	4.2084	5.2485
Cluster10	9.1729	9.0059	6.2857	7.7436	8.2350
Cluster11	9.2363	9.0365	6.3578	7.8487	8.2528
Cluster12	12.2006	11.6532	9.7681	9.8008	10.4034
Cluster13	9.2878	7.1436	4.3507	3.6947	4.8121
Cluster14	7.4258	5.5047	3.0617	3.1942	3.5615
Cluster15	8.1397	6.2447	3.6535	3.9146	4.2635
Cluster16	4.7594	8.7055	9.6853	10.3884	10.1355
Cluster17	5.8806	6.3339	7.1571	7.7978	7.3819
Cluster18	8.9075	8.5011	5.9257	7.3037	7.7279
Cluster19	8.8286	8.3833	5.6862	6.9307	7.4850
Cluster20	8.8938	8.3061	5.5957	6.6196	7.2778
Cluster21	9.3612	9.0405	6.5285	8.0058	8.4296
Cluster22	9.8505	9.3982	7.2970	8.1006	8.5331
Cluster23	10.4176	10.2050	7.9193	8.7053	9.5519
Cluster24	9.0391	6.7502	4.4770	3.7477	4.1969

	Cluster6	Cluster7	Cluster8	Cluster9	Cluster10
Cluster1	9.5013	8.1662	7.4917	8.2109	9.1729
Cluster2	7.0433	7.5016	6.3518	6.8870	9.0059
Cluster3	4.9580	5.2059	3.3162	3.6380	6.2857
Cluster4	3.4521	6.2695	4.3003	4.2084	7.7436
Cluster5	3.4993	6.7378	4.8873	5.2485	8.2350
Cluster6	0.0000	8.3925	5.9335	5.7667	9.2171
Cluster7	8.3925	0.0000	3.1302	3.8988	4.4774

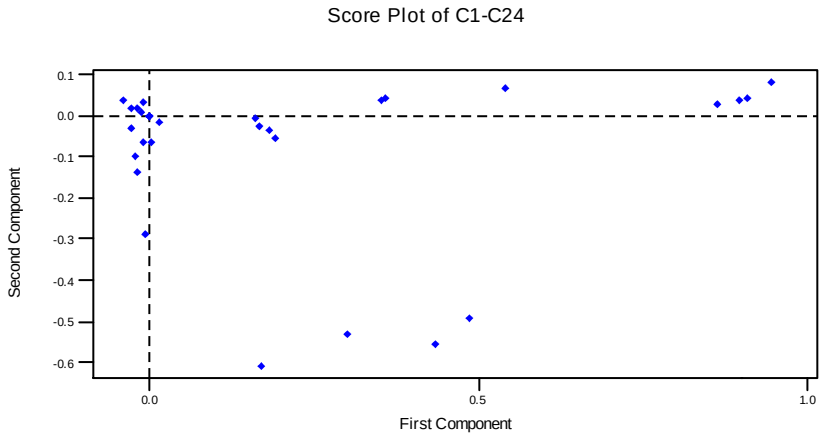
Cluster8	5.9335	3.1302	0.0000	1.8730	4.8522
Cluster9	5.7667	3.8988	1.8730	0.0000	4.8483
Cluster10	9.2171	4.4774	4.8522	4.8483	0.0000
Cluster11	9.1750	4.8850	5.1895	5.2576	1.0718
Cluster12	11.4185	9.7098	9.6420	9.3496	9.1797
Cluster13	3.9997	7.2745	5.0706	4.2636	7.8268
Cluster14	4.4270	5.6103	3.3355	4.1438	7.4197
Cluster15	4.2614	6.6157	4.3089	4.5987	7.8966
Cluster16	11.1292	10.3338	9.5514	10.5778	11.8753
Cluster17	8.6796	8.2368	7.0968	8.3183	10.0550
Cluster18	8.6866	5.0282	5.0160	5.1112	3.0911
Cluster19	8.5170	4.6397	4.6528	4.5797	2.4895
Cluster20	8.2373	5.0076	4.7688	4.5303	3.1868
Cluster21	9.3307	5.6511	5.7371	5.8138	3.3573
Cluster22	10.0130	6.2005	6.7773	6.8023	5.0639
Cluster23	10.6396	6.2211	6.4203	5.9898	5.7974
Cluster24	5.2910	6.8867	4.9943	4.7214	7.6581

	Cluster11	Cluster12	Cluster13	Cluster14	Cluster15
Cluster1	9.2363	12.2006	9.2878	7.4258	8.1397
Cluster2	9.0365	11.6532	7.1436	5.5047	6.2447
Cluster3	6.3578	9.7681	4.3507	3.0617	3.6535
Cluster4	7.8487	9.8008	3.6947	3.1942	3.9146
Cluster5	8.2528	10.4034	4.8121	3.5615	4.2635
Cluster6	9.1750	11.4185	3.9997	4.4270	4.2614
Cluster7	4.8850	9.7098	7.2745	5.6103	6.6157
Cluster8	5.1895	9.6420	5.0706	3.3355	4.3089
Cluster9	5.2576	9.3496	4.2636	4.1438	4.5987
Cluster10	1.0718	9.1797	7.8268	7.4197	7.8966
Cluster11	0.0000	9.0902	7.9335	7.5280	7.9717
Cluster12	9.0902	0.0000	10.3857	10.2556	10.6480
Cluster13	7.9335	10.3857	0.0000	4.0714	3.9351
Cluster14	7.5280	10.2556	4.0714	0.0000	2.8101
Cluster15	7.9717	10.6480	3.9351	2.8101	0.0000
Cluster16	11.8753	13.6507	11.7332	9.2075	10.0023
Cluster17	10.0699	12.2732	9.5516	6.5964	7.5775
Cluster18	3.1639	8.8211	7.3658	6.8915	7.4032
Cluster19	2.7137	8.4208	7.0893	6.6986	7.1863
Cluster20	3.2998	8.0904	6.7911	6.5638	6.9848
Cluster21	3.5203	8.9869	7.7527	7.4012	7.9325
Cluster22	5.3993	7.9673	8.6006	8.0219	8.5623
Cluster23	6.6899	9.4789	8.6887	8.4747	9.0230
Cluster24	7.8118	9.8181	4.2675	4.5855	4.3084

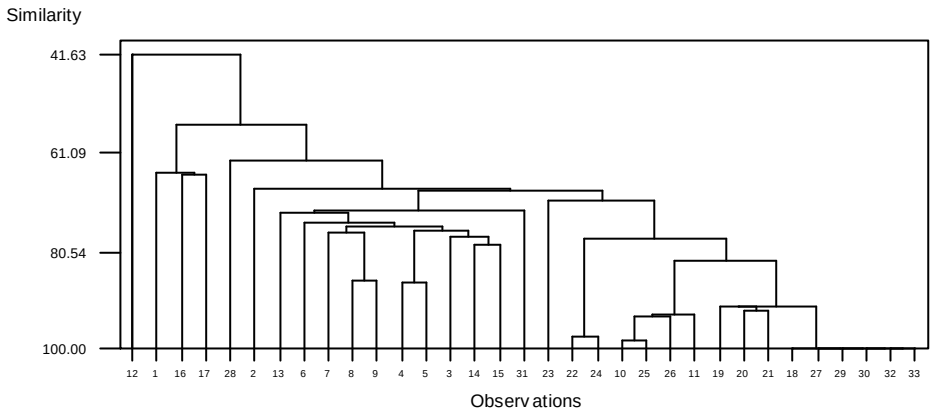
	Cluster16	Cluster17	Cluster18	Cluster19	Cluster20
Cluster1	4.7594	5.8806	8.9075	8.8286	8.8938
Cluster2	8.7055	6.3339	8.5011	8.3833	8.3061
Cluster3	9.6853	7.1571	5.9257	5.6862	5.5957
Cluster4	10.3884	7.7978	7.3037	6.9307	6.6196
Cluster5	10.1355	7.3819	7.7279	7.4850	7.2778
Cluster6	11.1292	8.6796	8.6866	8.5170	8.2373
Cluster7	10.3338	8.2368	5.0282	4.6397	5.0076
Cluster8	9.5514	7.0968	5.0160	4.6528	4.7688
Cluster9	10.5778	8.3183	5.1112	4.5797	4.5303
Cluster10	11.8753	10.0550	3.0911	2.4895	3.1868
Cluster11	11.8753	10.0699	3.1639	2.7137	3.2998
Cluster12	13.6507	12.2732	8.8211	8.4208	8.0904
Cluster13	11.7332	9.5516	7.3658	7.0893	6.7911
Cluster14	9.2075	6.5964	6.8915	6.6986	6.5638
Cluster15	10.0023	7.5775	7.4032	7.1863	6.9848
Cluster16	0.0000	4.7497	11.5143	11.4577	11.4232
Cluster17	4.7497	0.0000	9.6437	9.5740	9.5363
Cluster18	11.5143	9.6437	0.0000	1.1829	1.8815
Cluster19	11.4577	9.5740	1.1829	0.0000	1.0747
Cluster20	11.4232	9.5363	1.8815	1.0747	0.0000
Cluster21	11.9904	10.1956	3.4090	3.1126	3.4676
Cluster22	12.4306	10.7446	5.0146	4.3690	4.5425
Cluster23	12.8034	11.1249	6.1584	5.5301	5.7686
Cluster24	11.1839	8.7679	7.2896	7.0015	6.7870

	Cluster21	Cluster22	Cluster23	Cluster24

Cluster1	9.3612	9.8505	10.4176	9.0391
Cluster2	9.0405	9.3982	10.2050	6.7502
Cluster3	6.5285	7.2970	7.9193	4.4770
Cluster4	8.0058	8.1006	8.7053	3.7477
Cluster5	8.4296	8.5331	9.5519	4.1969
Cluster6	9.3307	10.0130	10.6396	5.2910
Cluster7	5.6511	6.2005	6.2211	6.8867
Cluster8	5.7371	6.7773	6.4203	4.9943
Cluster9	5.8138	6.8023	5.9898	4.7214
Cluster10	3.3573	5.0639	5.7974	7.6581
Cluster11	3.5203	5.3993	6.6899	7.8118
Cluster12	8.9869	7.9673	9.4789	9.8181
Cluster13	7.7527	8.6006	8.6887	4.2675
Cluster14	7.4012	8.0219	8.4747	4.5855
Cluster15	7.9325	8.5623	9.0230	4.3084
Cluster16	11.9904	12.4306	12.8034	11.1839
Cluster17	10.1956	10.7446	11.1249	8.7679
Cluster18	3.4090	5.0146	6.1584	7.2896
Cluster19	3.1126	4.3690	5.5301	7.0015
Cluster20	3.4676	4.5425	5.7686	6.7870
Cluster21	0.0000	4.0085	5.2691	7.9192
Cluster22	4.0085	0.0000	5.1086	8.4357
Cluster23	5.2691	5.1086	0.0000	8.5188
Cluster24	7.9192	8.4357	8.5188	0.0000



Dendrogram of samples from PCA



APPENDIX V C

Amalgamated distance and similarity levels in the high-resolution dendrogram graph in figure 5.28

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	32	100.00	0.000	32	33	32	2
2	31	100.00	0.000	30	32	30	3
3	30	100.00	0.000	29	30	29	4
4	29	100.00	0.000	27	29	27	5
5	28	100.00	0.000	18	27	18	6
6	27	98.19	0.248	10	25	10	2
7	26	97.43	0.351	22	24	22	2
8	25	93.61	0.872	10	26	10	3
9	24	93.24	0.923	10	11	10	4
10	23	92.38	1.041	20	21	20	2
11	22	91.67	1.137	19	20	19	3
12	21	91.33	1.183	18	19	18	9
13	20	86.77	1.806	4	5	4	2
14	19	86.28	1.873	8	9	8	2
15	18	82.44	2.397	10	18	10	13
16	17	79.41	2.810	14	15	14	2
17	16	78.02	3.000	10	22	10	15
18	15	77.57	3.062	3	14	3	3
19	14	77.07	3.130	7	8	7	3
20	13	76.60	3.194	3	4	3	5
21	12	75.71	3.316	3	7	3	8
22	11	74.71	3.452	3	6	3	9
23	10	72.93	3.695	3	13	3	10
24	9	72.55	3.748	3	31	3	11
25	8	70.64	4.008	10	23	10	16
26	7	68.40	4.313	3	10	3	27
27	6	68.17	4.346	2	3	2	28
28	5	65.21	4.750	16	17	16	2
29	4	65.13	4.759	1	16	1	3
30	3	62.58	5.109	2	28	2	29
31	2	55.49	6.075	1	2	1	32
32	1	41.63	7.967	1	12	1	33