MICROBIOLOGICAL, POLYCYCLIC AROMATIC HYDROCARBON AND HEAVY METAL CONTENT OF SOIL AND WATER SOURCES IN COMMUNITY AROUND PETROLEUM PRODUCTSDEPOT SULEJA, NIGERIA

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ABSTRACT

Contamination by petroleum products is a common occurrence in various petroleum depots in Nigeria. This study was aimed at assessing the microbiological, polycyclic aromatic hydrocarbons (PAHs) and heavy metal quality of soil and water sources in communities around Petroleum Products Marketing Company (PPMC), Suleja, Nigeria. Soil and water samples were collected from the petroleum depot (Plot 3) and the five communities designated as plots (TungaShanu - Plot 1, Dikko - Plot 2, Dagweru - Plot 4, Tunga Koro -Plot 5 and Maje - Plot 6) around the petroleum products depot and a control site. Microorganisms in the soil and water samples were enumerated by spread inoculation on general purpose media and selective media, characterized and identified using biochemical and molecular techniques. Bacterial and fungal isolates were tested for their potential to utilize petroleum products in a Bushnell Haas Broth containing 0.05 mL of petroleum products (diesel, kerosene, engine oil, crude oil) as sole source of carbon and energy. The utilization rate was determined by spectrophotometry and viable cell counts. The capacities of selected bacterial and fungal isolates to mineralize crude oil and PAHs were further tested in minimal salt medium. The soil and water samples were also analyzed for PAHs and heavy metals; Lead (Pb), Cadmium (Cd), Arsenic (As) Copper (Cu), Chromium (Cr), Manganese (Mn) to evaluate the potential contaminant contribution attributed to PPMC activities. The bacteria isolated were species of *Staphylococcus, Streptococcus, Proteus, Pseudomonas, Bacillus* and *Escherichia*; while the fungal isolates were *Penicillium, Aspergillus, Mucor*, and *Rhizopus*. Total coliform bacteria and hydrocarbon utilizing bacteria were also among the isolates identified. Soil samples had higher aerobic heterotrophic bacterial counts than the water samples (11.0 x 10^6 cfu/g – 62.0 x10^6cfu/g and 3.2 x10^4cfu/mL – 9.8 x10^4cfu/mL) respectively. Crude oil was most utilized by the microbial isolates than other petroleum products. *Pseudomonas* and *Bacillus* among bacteria had the highest capacity in utilizing the petroleum products while *Aspergillus* and *Penicillium* exhibited greater capacity among fungi. The total PAHs ranged from 4.00 - 428.00 mg/kg, 4.11 - 584.03mg/kg, 8.00 - 324.00mg/kg, 4.00 - 452.00mg/kg, 4.00 - 376.00mg/kg, and 8.05 - 388.00mg/kg for Plots 1,2,3,4,5 and 6 respectively in soil samples. Total PAHs concentration in water samples ranged from undetectable level to 0.3372 μg/mL in all the Plots. The mean concentration of the heavy metals in the soil for the various plots ranged from Cr 0.25-1.21 mg/kg, Cd 1.69-3.15 mg/kg, Pb 4.11-8.27 mg/kg, As 1.42-2.50 mg/kg, Mn 2.15-4.75, and Cu 1.10- 3.32 mg/kg. Mean concentration of the heavy metals in water samples for the various plots ranged from Cr 0.05-1.24 mL/L, Cd 0.01-1.95 mL/L, Pb 0.01-3.72 mL/L, As 0.00-2.24 mL/L, Mn 1.14-3.75 mL/L, and Cu 0.00-1.26 mL/L. Geo-accumulation indices showed heavy metals in the decreasing order; As(2.99) > Cr (1.07) > Cd (0.88) > Pb (0.63) > Cu(0.29) > Mn (0.14). The contamination factor (CF) and pollution load indices (PLI) were in the order: Pb > Cd > Cr > As > Cu > Mn. Present study revealed microbial isolates capable of utilizing the various petroleum products and PAHs and therefore can be useful in oil spill bioremediation in the tropical environment. The results also revealed that the concentration of some PAHs particularly Phenanthrene, Naphthalene and Benzo (b)fluoranthene and most of the heavy metals were higher than the permissible limits set by the World Health Organization and may constitute health challenges and ecological risks in the study areas.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Petroleum is a complex mixture of hydrocarbons and non-hydrocarbon compounds (Norman, 2001; Onyenekenwa, 2011). Petroleum products composed mainly of hydrocarbons of different types and traces of some potential toxic elements can contaminate soils and leach into ground and surface waters, which are major sources of water for drinking and domestic use and can further get into the food web, and be accumulated by plants and animals, including man. Pollution of the environment by petroleum products is an inevitable consequence of oil production, transportation and distribution activities. Crude oil, when refined contains a wide range of components such as hydrocarbons, heavy metals, dye additives, antioxidants and corrosion inhibitors (Ollivier and Magot, 2005; Akporido, 2008). The refined products show higher toxicity compared to crude oil since metal speciation is altered and new metals added to the matrix during the refining processes (Uzoekwe and Oghosanine, 2011). The waste generated from such processes may contain spent catalysts which are not recovered in most cases but discharged into soil and receiving water bodies where they accumulate in surface waters, sediments of rivers, and ultimately groundwater (Ekiye and Zejiao, 2010).

There are four petroleum product marketing company (PPMC) depots in various locations in Niger State. These include, Suleja, Izom, Minna (Pogo) and Sarki Pawa depots. Only two (Pogo and Suleja) out of these four depots are functional. These depots were originally in remote areas well suited for petroleum products marketing

company (PPMC) depot. However, due to rapid increase in population and urbanization, sparse of land around the depot are now being used for residential estates. The communities around these PPMC depots depend mostly on ground water and streams for their domestic water need. Environmental pollution has been associated with the activities of these PPMC depots. Often seen along these depots are long queue of trucks awaiting loading of petroleum products. These heavy trucks contribute to pollution of the environment as leakages and waste oils from broken down trucks are common sight in these areas. Ollivier and Magot (2005) and Akporido (2008) reported that oil spills or leakages from storage tanks and/or tanker trucks during loading and offloading of petroleum products or washing of oil storage tanks, damaged pipelines (old or vandalized) can cause soil environment to be contaminated.

The microbial transmission of infectious diseases via contaminated soil and water continues to be a risk to public health in Nigeria and all over the world. According to National Bureau of Statistics (2009), at least 27 % of Nigerians depend absolutely on streams, pond, river and rainwater for their drinking water source. Research has shown high prevalence of waterborne diseases such as cholera, diarrhoea, dysentery and hepatitis among Nigerians (Oguntoke *et al*., 2009; Raji and Ibrahim, 2011). Studies carried out in most cities in Nigeria have shown that industrial effluent is one of the main sources of surface water pollution in Nigeria (Olayinka and Alo, 2004; Esoka and Umaru, 2006; Ekiye and Zejiao, 2010; Emiola *et al*., 2010). Several bacterial genera and some parasites have been implicated in water related diseases. These include *Vibrio, Salmonella, Shigella, Escherichia, Campylobacter, Cryptosporidium,* Giardia, *etc.* (Hunter *et al*., 2009; Oyedum *et al*., 2016). Coliform bacteria are naturally occurring in soil and are found on vegetation and in surface waters. Though coliform bacteria do not

cause illness in healthy individuals, their presence in water bodies indicates the water system is at risk to more serious forms of contamination (Oyedum *et al*., 2016).

There are many petroleum derivatives important to man because of their activities. However, this study focused on polycyclic aromatic hydrocarbons (PAHs). Among the derivatives of petroleum, PAHs have been identified as recalcitrant organic pollutants. Chemically, PAHs are aromatic compounds formed by two or more benzene rings, constituted exclusively by carbon and hydrogen atoms, arranged in a linear, angular or grouped form (Arey and Atkinson, 2003). PAHs are residues of combustion, petroleum refinery and other industrial processes of high temperature (Bispo *et al*., 1999). PAHs are found in environmental medium such as air, soil and water (Kabziński *et al*., 2002). There are thousands of these substances in the environment, each one differing in the number and position of the aromatic ring (Eisler, 1987), but only 16 substances have been identified to cause severe health and environmental concern: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(ghi)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a, h)anthracene, phenanthrene, fuoranteno, fuoreno, indeno(1,2,3-cd)pyrene, naphthalene and pyrene (United States Environmental Protection Agency, USEPA, 1986).

PAHs have been implicated inskin, lung, bladder, liver,andstomach cancers in well-established animal model studies (Collins *et al.,* 1998). They are first chemically modified by enzymes into metabolites that react with DNA, which initiates mutations (Bostrom *et al*., 2002). Exposure to PAHs has also been linked with cardiovascular disease and poor fetal development (Bostrom *et al*., 2002). PAH can also act indirectly on organisms and cause alterations in the populations, since the increase in the density of the soil due to their presence and their hydrophobic properties decrease the

inhabitable space within the pores of the soil. Moreover, PAHs act as fungicides, eliminating the source of food of some organisms (Blakely *et al.,* 2002).

A huge bulk of waste generated from depot activities are oil discharges and since oil contains mostly hydrocarbons and heavy metals, these discharges have significantly impact on the pollution levels of underground and surface water near depots (Adeniyi and Afolabi, 2002; Manahan, 2003; Adewuyi *et al*., 2011). Water support all forms of biological resources (plant and animal life) and are normally obtained from two major natural sources which are surface water (water bodies) such as rivers, streams, fresh water lakes and ground water (geological water) such as borehole and well water (Vanloon and Duffy, 2005; Mendie, 2005). Water is capable of dissolving, absorbing, and adsorbing or suspending many different compounds (WHO, 2007) as well as contaminants from its surrounding and those arising from humans and animals as well as other biological activities due to its distinctive chemical properties (Mendie, 2005). Ground water contamination is one of the most essential environmental issues confronting mankind today (Vodela *et al*., 1997). Among the wide diversity of contaminants affecting water resources in recent time heavy metals receive particular concern as a result of their strong toxicity even at low concentrations (Olowu *et al.,* 2010; Majolagbe *et al*., 2011; Olowu *et al*., 2012).

The high toxic and persistent nature of heavy metals in the environment has made heavy metals priority pollutants. For good environmental management, an understanding of the changing concentration and distribution of heavy metals and their compounds in various compartments of the environment is of essence. Research on Nigerian crude oils revealed that they contain relatively high concentrations of some heavy metals, Fe, Zn, Cu, Pb and Hg (Kakulu, 1985). Several petroleum industries' production and processing

activities, including crude oil transformation to produce final consumable products such as liquefied petroleum gas (LPG), premium motor spirit (PMS), automotive gas oil (AGO), dual purpose kerosene (DPK), etc, result to the release of heavy metals, among others to the environment such as receiving waters (Nwafulugo *et al*., 2014). Pollution by heavy metals in terrestrial ecosystems has been recognized as a serious environmental concern, due to their non-biodegradability and tendency to accumulate in plants and animal tissues (Otitoloju *et al*., 2009). The contamination of soil by heavy metals leads to the decrease in the diversity of species due to changes in the composition of the community that eliminate the most sensitive species (Beyrem *et al*., 2007) and promote the tolerance of opportunistic species (Syrek *et al*., 2006) or invasive species (Piola and Johnston, 2008).

1.2   Statement of the Research Problem

The increase in the demand of petroleum products results in a corresponding increase in the production, refining, transportation to depot and to the final destinations of these products. These petroleum activities often result in the heavy pollution of the environment (Amokaye, 2004). Other sources of pollution are leakages from depot reservoirs and from tankers during and after loading of petroleum products within and around the depots. Communities have also interacted negatively with contact soil and water bodies (Oberdorster and Cheek, 2000). Long queue of trailer tankers (loaded and awaiting loading) are common sights in PPMC depots, and these are potential sources of petroleum product spills. Environmental consequences of soil pollution by petroleum products include adverse effect on soil microflora which play vital role in soil fertility and maintenance of healthy ecosystem. Petroleum contamination of soil and water may

result in gene mutation of microorganisms which is a great actor in nutrient cycle (Onwurah, 2000).

Communities around Suleja PPMC depot depend mostly on ground water for their domestic water need. These water sources are polluted by the activities of PPMC through spills, leakages, effluent water disposal and other sources. The influx of people within and around Suleja PPMC depots coupled with the irregular or unplanned settlement structures have resulted in high pollution of the environment by these individuals. Within these PPMC depot communities, indiscriminate defaecation and urination are often observed, and these are heavy sources of microbial contamination. This is due to the fact that, there are little or no proper sanitary structures in the area. Thus, open defaecation contaminates the soil, surface water and may seep into underground water through runoff or leachate. Microbial contamination of the soil also occurs through open baths at night by the truck drivers and accompanying persons which may be carried by erosion into surface and underground water where they may also cause more pollution. Contamination of soil by petroleum products could lead to a depression of microbial density and activities even in case of relatively light contamination (Odu, 1972). Petroleum products contaminated soil retards the activities of the nitrogen-fixing bacteria and may result in poor agricultural yields.

Although human health effects from environmental exposure to low levels of PAHs are unknown, exposure to high level of PAH is detrimental to health. The lipophilic nature of PAHs makes it easier to penetrate biological membranes and accumulate in organisms (Tuvikene, 1995). Large amounts of naphthalene in air can irritate eyes and breathing passages. Polycyclic aromatic hydrocarbons are potential human carcinogens and their mutagenicity and carcinogenicity have been experimentally reported

(Armstrong *et al*., 2004). Various bio-effects of PAHs have been properly demonstrated and documented on a large scale such as interactive effects on DNA and RNA; haematological parameters; hepatic lesions and changes; reproductive abnormalities; immune-suppression and developmental toxicity (Kurelec and Gupta, 1993; Everaarts *et al*., 1993; Incardona *et al*., 2006; Singh *et al*., 2008). Exposures to large amounts of naphthalene from skin contact with the liquid form and from breathing naphthalene vapor lead to blood and liver abnormalities. Due to poor institutional funding by the Nigerian governments, studies on the levels of PAHs and other organic contaminants in the country are very scanty despite PAHs being listed as priority pollutants that need to be monitored in both industrial and domestic effluents, soil and freshwater systems (Emoyan, 2009).

Heavy metal pollution may occur in the vicinity of PPMC depots due to damages, rupture or leakages from petroleum pipes, spills by tankers during loading and offloading of products, fossil fuel combustion, washing of oil storage tanks and deliberate discharge of effluents by refineries. All these discharges arising from activities in the depot significantly contaminate soils and ultimately pollute both surface and ground water through leaching or infiltration thereby causing serious health and environmental hazards. Lead is a constituent part of automobile fuel and has relatively low concentration in environment. It is not very soluble and hence nearly immobile in soil and very toxic to humans and organisms with long lasting effects. No level of lead is considered safe in drinking water although an action level of 15 µg/L (parts per billion) can be used to identify highly impacted water (Duruibe *et al*., 2007). Other heavy metals such as cadmium (Cd), copper (Cu), chromium (Cr), manganese (Mn), nickel (Ni) and zinc (Zn) which are also found in petroleum products are non-

degradable in the soil (Akguc *et al.*, 2008). The accumulation of heavy metals in agricultural soils is of increasing concern due to the food safety issues and potential health risks not only on crop plants but also on human health as well as its detrimental effects on soil ecosystems (Prabhat *et al.*, 2019). The use of available land near PPMC depots by depot settlers for farm land is a common practice, and polluted soil and crops in these areas could be potential sources of human exposure to contamination. The presence of priority heavy metals in soil and water and other media denotes pollution and provide overview of possible human exposure to the contaminant and consequent toxicity.

In Niger State, there is scanty or no information on the impact of petroleum and petroleum products in soil and water around Suleja depot and their surrounding communities. Therefore, there is need to provide adequate information about the microbiological, hydrocarbon and heavy metal qualities of soil and water sources in communities around petroleum products marketing company (PPMC) depot Suleja, in Niger State.

1.3   Justification for the Study

There are twelve storage tanks in Suleja depot in Niger State with each tank having an average capacity of eleven million two hundred thousand (11,200,000) litres. These storage tanks are prone to leakages and washing with their waste water released to the environment perhaps untreated. The PPMC depots are usually sited at the outskirts of town but due to urbanisation most PPMC depots in Nigeria are now in unplanned residential areas with heavy commercial activities. Communities around Suleja depot depend on available underground and surface water for their basic water need which may be contaminated by the depot activities. There is need therefore to periodically

evaluate the impact of these petroleum activities and their corresponding consequences to ensure a sustainable environment.

The influx of people within and around Suleja depot poses great concern to public health due to irregular and unplanned settlement pattern within these communities. There is high probability of microbial contamination of soil, surface and underground water as a result of open defaecation and urination within these depots communities. It is therefore essential to have a regular assessment of the microbial qualities of surface and underground water within these regions to ensure the safety of water.

Considerable numbers of Suleja depot dwellers are predominantly farmers that depend on soil, surface and underground water around for their farming activities. PAH reduces the performance of nitrification with an overall impact on crop production. Reactive metabolites of some PAHs such as epoxides and dihydrodiols, bind to cellular proteins and DNA. The resulting biochemical disruptions and cell damage lead to mutations, developmental malformations, tumours, and cancer. Evidence indicates that PAHs are carcinogenic to humans. Though the effects of PAHs on human health depend mainly on the length and route of exposure, the amount or concentration and of course the innate toxicity of the PAHs, they have been established to be carcinogenic, teratogenic, genotoxic and immunotoxic.A variety of other factors can also affect human health including subjective factors such as pre-existing health status and age. It is important therefore to assess and provide recent information on the level of PAH pollution within these depots community.

Heavy metals discharge from vehicles fumes, engine oil, wastes (hazardous or municipal), ruptured, damaged or leak oil pipes and accidental oil spillage from tanker which results in contamination of soil and water in Suleja depot in Niger state need to

be properly assessed. These heavy metals can accumulate in plant parts and be transferred to humans through food chain. Lead which is one of the priority heavy metals is a constituent of petroleum product and is released into the environment via petroleum related activities. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs (Kaye *et al*., 2002). Heavy metals can also contaminate ground water which is the major source of drinking water available to Suleja depot communities through leaching and erosion.

This study is therefore designed as an Environmental Impact Assessment (EIA) to provide information on the microbiological, hydrocarbon and heavy metal qualities of soil and water sources in communities around PPMC depots in Niger State.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of this study was to assess the microbioloical, polycyclic aromatic hydrocarbon and heavy metal content of soil and water sources in community around petroleum products marketing company depot Suleja, Niger State.

1.4.2 Objectives of the study

The objectives of this study were to:

i. Enumerate, characterise and identify microorganisms in soil and water sources in communities around petroleum product depot, Suleja.

ii. Determine the Polycyclic aromatic hydrocarbons (PAHs) in soil and water sources.

iii. Determine the heavy metals in soil and water sources.

iv.   Screen the microbial isolates for potential to utilize petroleum products and PAH.

 v.   Determine the gene responsible for PAH degradation in selected microbial isolates.

CHAPTER TWO

2.0   LITERATURE REVIEW

2.1   Microbial Qualities of Water Sources

In many parts of Nigeria as well as other developing countries, treated pipe borne water is limited and often inadequate for the teeming human population, thus, an increasing number of people in semi-urban and urban areas in Nigeria depend on surface and underground water for their daily water need (Idowu *et al*., 2011). Water is essential for the survival of every form of life and the need for water is constantly increasing due to high rates of population growth and urbanization. However, the increased demands of water for domestic, agricultural and industrial purposes are not commensurate with water availability thus, posing significant risks in maintaining acceptable water quality (Dara, 1993; Gopinath *et al*., 2012).

Bacterial contamination of drinking water is a major public health problem worldwide, because this water can be an important vehicle of diarrheal diseases, thus, the need to evaluate the microbiological quality (Suthar *et al.,* 2009). Monitoring the microbiological quality of water is done through laboratory testing for the coliform group. The term "total coliforms" refers to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. The group includes thermo-tolerant coliforms which are of faecal origin, found in the intestines of humans and warm-blooded animals, as well as the environmental species that are naturally found on plants, soil, and water. The thermo-tolerant coliforms are widely used as microbiological parameters indicating faecal contamination while total coliforms serve as a parameter to provide basic information on water quality (Shibata *et al*., 2004). The need to assess the quality

of surface and underground water is imperative because they have direct effects on the health of individuals.

The presence of faecal coliform in water may indicate that the water has been contaminated with faecal material of humans or other animals. Groundwater in a properly constructed well should be free of coliform bacteria. Faecal coliform bacteria can be found in wells through direct discharge of waste from mammals and birds, agricultural and human sources. This could also happen due to poor construction or cracks in the well or through runoffs from wooded areas, pastures, feedlots, septic tanks, and sewage plants into streams or groundwater (Akinyemi, *et al*., 2006; Oyedum *et al*., 2016). The use of soakaways for the disposal of domestic and industrial effluents and even citing of refuse dumps for both domestic and industrial solid wastes may impair groundwater quality unless there is an impermeable stratum between the disposal area and the groundwater table. Ademoroti (1987) reported the contamination of well water by *Vibrio cholerae* and coliform bacteria from many Nigerian cities and villages and recommended that a minimum of 30 m must seperate a well from a soak-away site. Elimian *et al*. (2019) reported atotal of 43996 cholera cases and 836 cholera related deaths in 20 states in Nigeria in 2018 cholera outbreak and suggested a multi-disciplinary approach aimed at preventing and controlling cholera in Nigeria. Okello *et al*. (2019) also reported a cholera outbreak in eastern Uganda in 2016, caused by drinking contaminated surface and underground water and suggested boiling or treating of drinking water and restrictions on washing cloths near drinking water collection points.Regular monitoring of the microbial quality of drinking water is an essential step in ensuring the safety of drinking water (Pillai *et al*., 1997).

2. 2   Effects of Microbial Contamination of Water

Water remains the major source of transmission of enteric pathogens in developing countries. Notified cases are mostly in children especially those under 5 years of age (Federal Ministry of Health, Nigeria, 1991), in whom gastroenteritis usually manifests as acute diarrhea and often may require hospitalization. The conditions are usually less severe in adults and may resolve without serious medical attention. In Nigeria, there is a high incidence of childhood diarrhea despite the intensive activities of the National Diarrhea Control Program. This is due to the unavailability of potable water especially in rural communities, and mothers usually obtain water from unhygienic sources for preparing weaning foods (Izah and Ineyougha, 2015). Water availability is a major concern in most PPMC depot communities in Nigeria because most depots were built in outskirts of town without settlement plans. Studies across the country have shown that viruses, bacteria, protozoa, and helminthes are variously responsible for diarrhea diseases in children (Okeke *et al*., 2003; Alabi *et al*., 1998). Water pollution affects plants and organisms living in these bodies of water. In almost all cases the effect is damaging not only to individual and populations, but also to the natural biological communities.

The high values of coliform reported for some river water samples confirm faecal pollution from domestic sewage, dumping sites, abattoir activities, etc. High coliform values are typical characteristics of many water bodies in Nigeria. For instance, high population of faecal coliform counts has been reported in Ikpoba River, Edo state of Nigeria by Tatah and Ikenebomeh (1999). Similarly, Nwankwu (1992) reported coliform values in the range of 3,100-150,000 cfu 100 mL^{-1} at Iddo area of the Lagos lagoon. The high population of these microbial pollutants was linked to contamination

from the dumpsites. Pollution of surface and underground water in depot communities in Nigeria therefore calls for great attention. Some people regard water body as medium for waste disposal. Faecal pollution of water bodies in Nigeria signifies poor sanitation management as well as unhygienic manner of living among people and must be controlled to avoid outbreak of diseases.

2.3   Water Related Diseases

Water safety and quality are fundamental to human development and well-being. The occurrence of water related diseases in developing countries has been on the increase over the decades largely due to unsafe water, inadequate sanitation, water and poor hygiene among human population (Ali *et al*., 2011). Nigeria is one of the countries suffering from the devastating effects of water related diseases. Contaminated water and poor sanitary conditions increase the vulnerability to water related diseases. The distribution of water on earth varies from place to place, which brings about its scarcity and thus water related diseases. Water related diseases are caused by pathogenic microorganisms which are transmitted in contaminated fresh water (Kumar and Harada, 2002; Figueras and Borrego, 2010). Only 26.5 percent of the world's population has access to improved drinking water sources and sanitation facilities (WHO, 2010).

Poor access to improved water and sanitation in Nigeria remains a major contributing factor to high morbidity and mortality rates among children under five. About 200,000 Nigerian children are said to die annually of water-related diseases while 23.5 per cent of the population defecate in the open (WHO, 2010).

2.3.1   Classification of water related diseases

Water related diseases are classified into four main groups: (Magana-Arachchi and Wanigatunge, 2020):

i.   **Water-borne microbiological diseases**: The infections are spread through contaminated drinking water.Water borne diseases are caused by various bacteria, virus, protozoa and pathogenic microorganisms and usually occur as a result of poorly treated drinking water and wastewater or a natural disaster, like flooding and environmental pollutants (Patel*et al*., 2016).The common water-borne diseases in Nigeria include Cholera, Dracunculiasis, Hepatitis, Typhoid and Filariasis.

ii.   **Water-washed diseases**: These diseases arise due to the lack of proper sanitation and hygiene. The incidence, prevalence or severity of these diseases whose can be reduced by using safe (clean) water to improve personal and domestic hygiene. They include; Scabies, Shigellosis; Trachoma (Magana-Arachchi and Wanigatunge, 2020).

iii.   **Water-based diseases**: The infections are transmitted through an aquatic invertebrate. They are caused by skin contact with pathogen infested water or with chemical contaminated water. These include; Schistosomiasis (bilharzia); cyanobacteria caused disease.

iv.   **Water-related vector-borne diseases**: These diseases are transmitted by insects that depend on water for their propagation. In this type of water related diseases, the vector lives all or part of its life in or adjacent to a water habitat. The diseases and their vectors include; Malaria (mosquitoes); Filiariasis (mosquitoes); Onchocerciasis (aquatic flies); Schistosomiasis (snails); Trypanosomiasis (tsetse flies)

v. **Waterborne chemical disease:** The Disease is caused by ingestion of toxic substances in water from treated or untreated (raw) water or public (municipal)/private water supply. Common example of waterborne chemical disease arsenicosis (Gibson, 2014).

2.4   Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants of over 100 different chemicals that are formed during the incomplete combustion of plant or animal matter, or carbon fuels, such as coal or petroleum (Latimer and Zheng, 2003; Baklanov *et al.*, 2007). Polycyclic aromatic hydrocarbons are organic compounds which are mostly colourless, white, or pale-yellow solids. These chemically related compounds are environmentally persistent and possess various structures and varied toxicity. They have toxic effects on organisms through various actions (Bostrom *et al.*, 2002).

PAHs are characterized with high melting and boiling points thus, they are solids. They also have low vapor pressure, and very low aqueous solubility (Masih *et al.*, 2012). The latter two characteristics tend to decrease with increasing molecular weight, on the contrary, resistance to oxidation and reduction increases (Masih *et al.*, 2012). Aqueous solubility of PAHs decreases for each additional ring (Masih *et al.*, 2010). Meanwhile, PAHs are very soluble in organic solvents because they are highly lipophilic. PAHs also manifest various functions such as light sensitivity, heat resistance, conductivity; emission ability, corrosion resistance, and physiological action (Akyuz and Cabuk, 2010). PAHs possess very characteristic UV absorbance spectra. Each ring structure has a unique UV spectrum; thus, each isomer has a different UV absorbance spectrum. This is especially useful in the identification of PAHs. Most PAHs are also fluorescent,

emitting characteristic wavelengths of light when they are excited (when the molecules absorb light) (Masih *et al*., 2012).

PAH emissions from anthropogenic activities predominate; however, some PAHs in the environment originate from natural sources such as open burning, natural losses or seepage of petroleum or coal deposits, and volcanic activities. Major anthropogenic sources of PAHs include residential heating, coal gasification and liquefying plants, carbon black, coal-tar pitch and asphalt production, coke and aluminum production, catalytic cracking towers and related activities in petroleum refineries as well as motor vehicle exhaust (Juhasz and Naidu, 2000; Ravindra *et al*., 2008; Ramesh *et al*., 2011). Automobile exhaust, industrial emissions and smoke from burning wood, charcoal and tobacco contain high levels of PAHs. In general, more PAHs form when organic materials burn at low temperatures, such as in wood fires or cigarettes (Pitter and Chudoba, 1990). High-temperature furnaces produce fewer PAHs. Smoke from fires contains tiny particles of PAHs and other chemicals. These are known as particulate matter (PM). When PM is microscopic in size, it remains suspended in air and can move long distances.

PAHs are found in the ambient air in gas-phase and as sorbet to aerosols. Atmospheric partitioning of PAH compounds between the particulate and the gaseous phases strongly influences their fate and transport in the atmosphere and the way they enter into the human body (Wang *et al*., 2013). Most PAHs do not dissolve in water but, instead, bind to sediments. When sediments become suspended in water, PAHs can be transported with the sediment. PAHs can enter groundwater from ash, tar, or creosote that is improperly disposed in landfills and cause pollution (Wong *et al*., 2002). Many PAHs have toxic, mutagenic and/or carcinogenic properties. PAHs are highly lipid soluble and thus are readily absorbed from the gastrointestinal tract of mammals. They are rapidly

distributed in a wide variety of tissues with a remarkable tendency for localization in body fat. Metabolism of PAHs occurs via the cytochrome P450-mediated mixed function oxidase system with oxidation or hydroxylation as the first step. PAHs effects have been documented on immune system development, humoral immunity and on host resistance (Armstrong *et al*., 2004; Canadian Council of Ministers of the Environment, CCME, 2010). Structures of some PAH compounds are presented in Figure 2. 1.
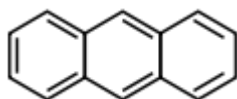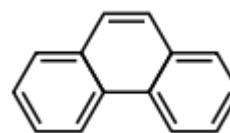
Anthracene   **Phenanthrene**
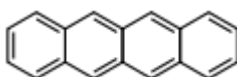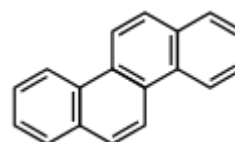
Tetracene   **Chrysene**
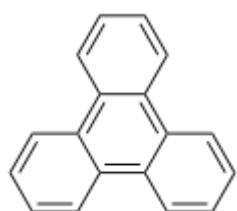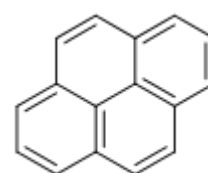
Triphenylene   **Pyrene**
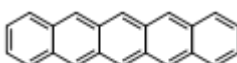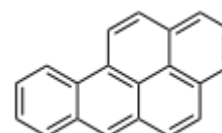
Pentacene   **Benzo[a] pyrene**

Corannulene
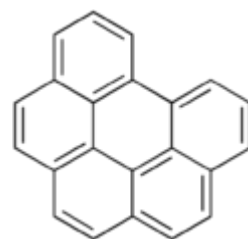
Benzo (g,h,i) perylene
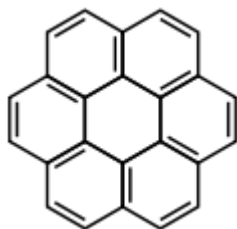
Coronene

Ovalene
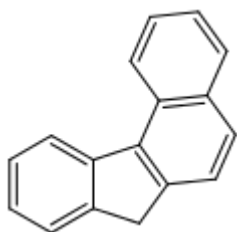
Benzo(c) fluorene

Figure 2.1:Structures of Some Common PAH Compounds

Source: Lundstedt (2003)

2.4.1 Sources of PAH and its transportation in the atmosphere

Three major sources of PAHs to the environment have been identified as follow; petrogenic, biological and pyrogenic sources. The mode of PAHs formation can be either natural or anthropogenic (Tolosa *et al*., 1996). Whenever organic substances are exposed to high temperatures under low oxygen or no oxygen conditions, pyrogenic PAHs are formed through a process known as pyrolysis. The destructive distillation of coal into coke and coal tar, or the thermal cracking of petroleum residuals into lighter hydrocarbons are pyrolytic processes which occur intentionally. Several other unintentionally processes occur during the incomplete combustion of motor fuels in cars and trucks, the incomplete combustion of wood in forest fires and fireplaces, and the incomplete combustion of fuel oils in heating systems. The temperature at which the pyrogenic processes occur ranges from about 350 °C to more than 1200 °C. Pyrogenic PAHs are generally found in greater concentrations in urban areas and in locations close to major sources of PAHs (Wang *et al*., 2011). In addition, PAHs can also be formed at lower temperatures. It is worth mentioning that crude oils contain PAHs that formed over millions of years at temperatures as low as 100–150 °C (WHO, 2003).

PAHs formed during crude oil maturation and similar processes are called petrogenic PAHs. Petrogenic PAHs are common due to the widespread transportation, storage, and use of crude oil and crude oil products (Tolosa *et al*., 1996). Some of the major sources of petrogenic PAHs include oceanic and freshwater oil spills, underground and above ground storage tank leaks, and the accumulation of vast numbers of small releases of gasoline, motor oil, and related substances associated with transportation (Tolosa *et al*., 1996; Ravindra *et al*., 2008). Researches have also shown that PAHs can be formed during the incomplete combustion of organic substances (Wang and Frenklach, 1997).

PAHs can be produced biologically, for example, they can be synthesized by certain plants and bacteria or formed during the degradation of vegetative matter (Masih and Taneja, 2006).

2.4.2 Deposition and distribution of PAHs in the soil

Through dry or wet deposition processes, atmospheric PAHs are continuously deposited to the earth. Some of these PAHs are from nearby sources, such as vehicle exhaust from adjacent roadways. PAHs may come from more distant sources and may be carried through various distances in the air. Some PAHs can be added to the soil if fill materials contain PAHs. When PAHs are deposited onto the earth's surface, they can become mobile since the majority of hanging PAHs in the soil will be bound to soil particles (Masih and Taneja, 2006, Cachada *et al*., 2012). The most important factors influencing PAH mobility of particulates in the subsurface are sorbent particle size and the pore throat size of the soils. Such pore throat can be defined as the smallest opening found between individual grains of soil (Riccardi *et al.,* 2013). If particles to which PAHs are sorbed cannot move through the soil then the movement of PAHs will be limited because they tend to remain sorbed to particles.

The tendency of PAH to be sorbed to soil depends on both the properties of the PAH and the soil. Therefore, PAH sorption is one of the processes that govern the soil mobility of individual PAHs. Numerous studies of the correlation of the partition coefficient with soil properties have found that the organic carbon content usually yields the most significant correlation (Electronic Power Research Institute, EPRI, 2000; USEPA, 2008; Moncada, 2016; Beriro, 2020).

2.4.3   Metabolism of PAHs by microorganisms

PAHs are relatively stable and recalcitrant in soils and less easy to degrade than many other organic compounds. They are difficult to remove from contaminated soil using the treatments that have been used successfully to clean soils contaminated with more degradable or volatile organic compounds such as alkanes (Pitter and Chudoba, 1990).Naphthalene has often been used as a model compound to investigate the ability of bacteria to degrade PAHs because it is the simplest and the most soluble PAH (Goyal and Zylstra, 1997). Therefore, information of bacterial degradation of naphthalene has been used to understand and predict pathways in the degradation of three- or more ring PAHs. Several bacteria genera have been isolated which utilize naphthalene as a sole source of carbon and energy. These include *Alcaligenes*, *Burkholderia*, *Mycobacterium*, *Polaromonas*, *Pseudomonas*, *Ralstonia*, *Rhodococcus*, *Sphingomonas*, and *Streptomyces* (Cerniglia, 1992; Kim *et al*., 2003; Ogbonna *et al*., 2012; Mohapatra and Phale, 2021).

Degradation of naphthalene starts through the multicomponent enzyme, naphthalene dioxygenase which attacks the aromatic ring to form *cis*-(1R, 2S)-dihydroxy-1, 2-dihydronaphthalene (*cis*-naphthalene dihydrodiol) (Simon *et al*., 1993; Goyal and Zylstra, 1997). The *cis*-naphthalene dihydrodiol formed by naphthalene dioxygenase is subsequently dehydrogenated to 1, 2-dihydroxynaphthalene by a *cis*-dihydrodiol dehydrogenase (Goyal and Zylstra, 1997). Subsequently, 1,2-dihydroxynaphthalene is metabolized to salicylate via 2-hydroxy-2*H*-chromene-2-carboxylic acid, *cis-o*-hydroxybenzalpyruvate, and 2-hydroxy-benzaldehyde (Denome *et al*., 1993; Baboshin *et al*., 2008). Also, 1,2-dihydroxynaphthalene is nonenzymatically oxidized to 1,2-

naphthaquinone (Goyal and Zylstra, 1997). Metabolic pathway of naphthalene by bacteria is shown in Figure 2.2.

The bacterial degradation of naphthalene has been well characterized for the catabolic enzyme system encoded by the plasmid NAH7 in *Pseudomonas putida* G7 (Denome *et al*., 1993). NAH7 has two operons that contain the structural genes for naphthalene degradation. One operon contains the gene for the upper catabolic pathway encoding the enzymes necessary for the conversion of naphthalene to salicylate. The second operon contains the gene for the lower catabolic pathway encoding the enzymes necessary for the metabolism of salicylate through the catechol meta-cleavage pathway to pyruvate and acetaldehyde (Denome *et al*., 1993; Goyal and Zylstra, 1996; Goyal and Zylstra, 1997). The entire sequence structure of plasmid NAH7 was determined by Sota *et al*. (2006).

2.4.4   Metabolism of PAHs in higher animals

Due to the high lipophilicity of this class of compounds, their bioavailability after ingestion and inhalation is significant. Scientific investigations have shown that detectable levels of PAH occur in almost all internal organs, particularly in organs that are rich in adipose tissue (International Programme on Chemical Safety,IPCS, 1998). These organs can serve as storage depots from which the hydrocarbons can be gradually released. Once they enter the organism polycyclic aromatic hydrocarbons require a multistep metabolic activation by specific enzymes. The enzyme system which is primarily responsible for PAH metabolism is the mixed-function oxidase system. The first reaction is an epoxidation. PAH epoxides can then be conjugated with glutathione and this is regarded as a true detoxification reaction (International Agency for Research on Cancer (IARC), 1983). The epoxides that are not conjugated with glutathione are

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converted into phenols and diols. These PAH metabolites, however, are sometimes not sufficiently polar to be excreted and therefore have to be conjugated with glucuronic or sulfuric acids to enable excretion. Most metabolites of PAH are excreted in faeces and urine (Hemminki *et al*, 1997).
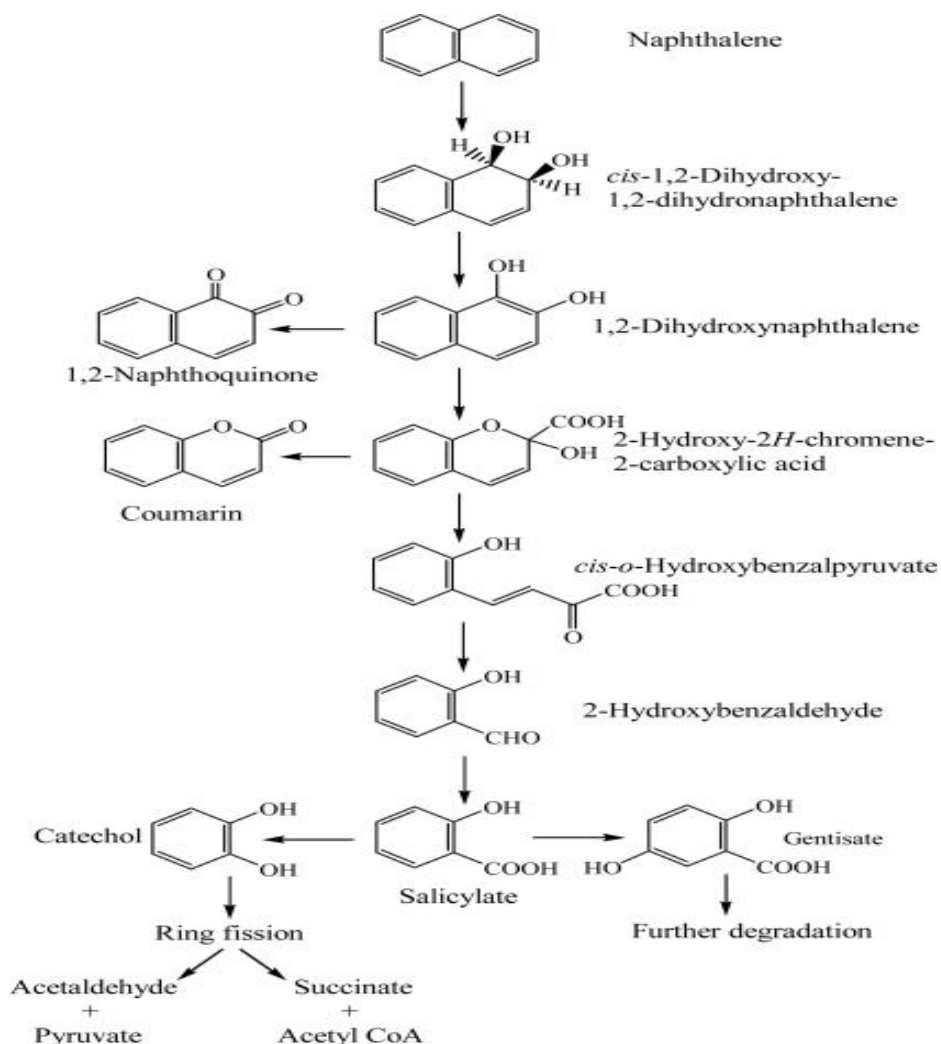
Figure 2.2: Metabolic Pathway of Naphthalene Degradation by Bacteria
Source: Auger *et al*. (2008)

2.4.5 Environmental consequences and ecotoxic effects of PAHs

PAHs are usually released into the air, or they evaporate into the air when they are released to soil or water. PAHs often adsorb to dust particles in the atmosphere, where they undergo photo oxidation in the presence of sunlight, especially when they are

adsorbed to particles. This oxidation process can break down the chemical constituents over a period of days to weeks (Igwe and Ukaogo, 2015). Due to the insoluble nature of PAHs in water, they are generally found adsorbed on particulates and precipitated in the bottom of water bodies or solubilized in any petroleum products where they easily contaminate water, sediments, and soil. However, mixed microbial populations in water and soil contribute in the degradation of some PAHs over a long period of time (Peter, 2003).

The toxicity of PAHs to aquatic organisms is affected by metabolism and photo-oxidation, and they are generally more toxic in the presence of ultraviolet light. PAHs have moderate to high acute toxicity to aquatic life and birds (Hussein e*t al*., 2016). PAHs in soil are unlikely to exert toxic effects on terrestrial invertebrates, except when the soil is highly contaminated. Adverse effects on these organisms include tumors, adverse effects on reproduction, development, and immunity (Collins *et al.,* 1998). Mammals can absorb PAHs by various routes such as; inhalation, dermal contact, and ingestion.

PAHs are moderately persistent in the environment and can bio-accumulate in some organisms. The concentrations of PAHs found in fish and shellfish are expected to be much higher than in the environment from which they were taken. Bioaccumulation has been also shown in terrestrial invertebrates (IPCS, 1998).

2.4.6   Effects of PAHs on human

The ecological as well as human health risks of PAHs are attracting serious concern due to their carcinogenic, mutagenic, as well as its persistent characteristics in the environment (Peng *et al*. 2011; Abdel-Shafy and Mansour 2016; Jia *et al*. 2017; Lasota and Błońska 2018; Cao *et al*. 2019). The effect of PAHs on human health may be described as acute/short-term health effects or chronic/long- term health effects. The effects on human depend mainly on the length and route of exposure, the amount or concentration of PAHs exposed to, and the innate toxicity of the PAHs (Menzie *et al*, 1992). A variety of other factors can also affect health impacts including subjective factors such as pre-existing health status and age. Mixtures of PAHs are known to cause acute skin irritation and inflammation. Anthracene, benzo(a)pyrene and naphthalene are direct skin irritants while anthracene and benzo(a)pyrene are known to cause an allergic skin response in animals and humans (International Programme on Chemical Society, IPCS, 1998).

Health effects from chronic or long-term exposure to PAHs may include decreased immune function, cataracts, kidney and liver damage (e.g. jaundice), breathing problems,asthma-like symptoms, and lung function abnormalities, and repeated contact with skin may induce redness and skin inflammation (Agency for Toxic Substances and Disease Registry, ATSDR, 1995). Naphthalene can cause the breakdown of red blood cells if inhaled or ingested in large amounts. Other traits possessed by PAHs include; carcinogenicity, teratogenicity, genotoxicity and immunotoxicity (IPCS, 1998; Canadian Council of Ministers of the Environment, CCME, 2010).

Ibe *et al*. (2021) carried out ecological risk assessment of the levels of polycyclic aromatic hydrocarbons in soils of the abandoned sections of Orji Mechanic Village, Owerri, Imo State, Nigeria andreported a total cancer risk above 1×10^{-6}, indicating a greater risk of cancer due to contact with the contaminated soil samples.This result is a serious ecological concern since the contaminated site was also used for agricultural activities. Long-term exposure to PAH pollutants could lead to severe problems such as brain dysfunction, cancer, tumors, nervous system, and reproductive disorders in humans (Kinawy,2009). Moreso, the findings of Nwaichi *et al*. (2017) from two oil and gas flaring impacted communities in Southern Nigeria revealed that study food crops from exposed areas were markedly contaminated by carcinogenic PAHs and pose public nutrition and health concerns in those communities.

2.5 Heavy Metals

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. Heavy metals may also be defined as metallic elements that have a relatively high density compared to water.Heavy metals exist in various forms in natural water.Heavy metals have found wide applications in industries, homes, agriculture, medicine and technology, and these applications have led to their wide distribution in the environment; raising serious concerns over their potential effects on microorganisms, human health and the environment (He *et al*., 2005). Metalloid such as arsenic is able to induce toxicity at low level of exposure and is also classified as heavy metal (Duffus, 2002). In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. Also, human exposure has risen dramatically as a result

of an exponential increase of their use in several industrial, agricultural, domestic and technological applications (Bradl, 2002, Narjala, 2020).

Heavy metals are also considered as trace elements because of their presence in trace concentrations (parts per billion range to less than 10parts per million) in various environmental matrices (Kabata- Pendia, 2001). Their bioavailability is influenced by physical factors such as temperature, phase association, adsorption and sequestration. It is also affected by chemical factors that influence speciation at thermodynamic equilibrium, complexation kinetics, lipid solubility and octanol/water partition coefficients (Hamelink *et al.,* 1994). Biological factors such as species characteristics, trophic interactions, and biochemical/physiological adaptation, also play an important role.

The essential heavy metals exert biochemical and physiological functions in plants and animals. They are important constituents of several key enzymes and play important roles in various oxidation-reduction reactions (WHO, 1996). Copper for example serves as an essential co-factor for several oxidative stress-related enzymes including catalase, superoxide dismutase, peroxidase, cytochrome c oxidases, ferroxidases, monoamine oxidase, and dopamine β-monooxygenase (Harvey and McArdle, 2008; Stern, 2010).

Researches in biological systems reveal that heavy metals affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair (Wang and Shi, 2001). Metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis (Chang *et al*., 1996; Wang and Shi, 2001).   Several studies from the laboratory have demonstrated that

reactive oxygen species (ROS) production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as arsenic (Tchounwou *et al*., 2001; Yedjou and Tchounwou, 2007), cadmium, chromium (Patlolla *et al*., 2009), lead (Patlolla *et al*., 2009), and mercury (Sutton *et al*., 2002; Sutton and Tchounwou, 2007). Based on their high degree of toxicity, these five elements rank among the priority metals that are of great public health significance. They are all systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. According to the United States Environmental Protection Agency (USEPA), and the International Agency for Research on Cancer (IARC), these metals are also classified as either "known" or "probable" human carcinogens based on epidemiological and experimental studies showing an association between exposure and cancer incidence in humans and animals (Yedjou and Tchounwou, 2007, WHO, 2011a).

Heavy metal-induced toxicity and carcinogenicity involves many mechanistic aspects, some of these mechanisms are not clearly understood. However, each metal is known to have unique features and physico-chemical properties that confer to its specific toxicological mechanisms of action (Hamelink *et al*., 1994).

2.5.1 Effects of heavy metals on human health

Heavy metals are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic, and teratogenic effects when accumulated beyond certain levels in the body (Obi *et al*., 2006). Some heavy metals can directly influence behavior by impairing mental and neurological function, influencing neurotransmitter production and utilization, and altering numerous metabolic body processes (Brodkin *et al*., 2007, White *et al*., 2007). Accumulation of heavy metal, even at levels well below those considered nontoxic, can have serious health effects (Lanphear *et al.,* 2005). Much of the damage produced by

toxic metals stems from the production of oxidative free radicals, which in turn cause lipid perioxidation of biomolecules and fragmentation of deoyribonuclei acid (Flora *et al*., 2007). Heavy metals disrupt a vast array of metabolic processes. They also alter pro-oxidant/antioxidant balance and bind to free sulfhydryl groups, resulting in inhibition of glutathione metabolism, numerous enzymes and hormone function. Nutritionally, heavy metals are directly antagonistic to essential trace elements and compete with nutrient elements for binding sites on transport and storage proteins, metalloenzymes and receptors. Disruption of the metabolism and balance of nutrient elements results in marked aberrations in the metabolism of carbohydrate, protein/amino acids, lipids, neurotransmitters, and hormones (Zalups, 2000).

The biological half-lives for heavy metals are variably long; the half-life for cadmium in the kidney is decades. Most heavy metals are readily transferred across the placenta, found in breast milk, and are well known to have serious detrimental effects on behaviour, intellect, and the developing nervous system in children (Sanders *et al*., 2009). Heavy metals can also increase the acidity of the blood and in effect, causes the body to draw calcium from the bones to help restore the proper blood pH. Furthermore, toxic metals set up conditions that lead to inflammation in arteries and tissues, causing more calcium to be drawn to the area as a buffer, contributing to hardening of the artery walls with progressive blockage of the arteries and osteoporosis (Satarug *et al.,* 2006). Even minute levels of toxic elements produce negative health consequences, affecting nutritional status, metabolic rate, the integrity of detoxification pathways, and the mode and degree of heavy metal exposure. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats, result to chronic renal failure and rheumatic heart disease.

Some heavy metals like lead and cadmium could adversely affect the male reproductive system; either by causing hypothalamic-pituitary axis disruption or by directly affecting spermatogenesis, resulting in impaired semen quality (Wyrobek *et al*., 1997; Jurasoviæ *et al*., 2004). Several studies have reported declines in semen quality associated with both lead and cadmium concentrations in blood (Adedoyin and Adesoye, 2005; Telisman *et al.,* 2007). Cadmium is also a known risk factor for osteoporosis, reportedly causing osteomalacia followed by osteoporosis at high concentrations. Cadmium is associated with diabetes and cancer (Schwartz *et al*., 2003).

2.5.2   Heavy Metal Poisoning in Nigeria

In March 2010, a team from Médecins Sans Frontière*s* (MSF, also known as Doctors Without Borders), monitoring endemic diseases such as meningitis and malaria, visited villages in Zamfara State (North Western Nigeria) after being informed by the local community of high mortality in children under five years old. MSF discoveries in these villages showed children's fresh graves and a mortality rate of more than 40 percent. The initial blood samples analysed for heavy metal poisoning from children and adults revealed high levels of lead in the blood — above 100 micrograms per deciliter (μg/dL). Centre for Disease Control (CDC) in Zamfara State thereafter analysed blood samples from a total of 205 children under the age of five from two villages. About 97 percent of the children in this group had lead levels above 45 μg/dL, the threshold for initiating chelation therapy, with some as high as 700 μg/dL. CDC's "level of concern"is just 10μg/dL. This unprecedented lead poisoning epidemic in Zamfara State, Nigeria, is the result of artisanal gold mining operations, where families participate in the gold processing even at homes.

March 9th 2015, 28 children (17 female and 11 male) below the age of five in Shikira village of Madaka District, Rafi local government area of Niger State were reported dead (International Centre for Investigative Reporting, ICIR, 2016). The villagers had termed the deaths mysterious especially because some of the affected children suffered convulsion and weakness of limbs. Medical examination however proved that the children had died from lead poising arising from illegal artisanal gold mining activities in the area. The 28 children who died were out of the 65 confirmed cases. Livestock including chickens, goats, cattle and sheep were also affected (ICIR, 2016). The World Health Organisation (WHO) blamed the deaths of the 17 female and 11 male children on high lead levels in the serum, 17-22 times in excess of acceptable limits (WHO, 2015). The result was further established from Medecins Sans Frontiers' specialised laboratory for detecting blood lead levels, thus forming the basis to solve the purported mystery of the strange ailment that had already killed 28 in the area (ICIR, 2016).

The effects of the lead poisoning were more devastating in Angwan Kawo village in Niger State because the village is located downstream of a tributary of the stream belt where mining of gems and gold and washing took place. It was reported that the gems and gold when mined, were washed in the stream which flowed down to the village that used the water from the stream for domestic purposes (ICIR, 2016). Lead poisoning is generally characterised by abdominal pain, headache, anaemia, irritability, seizures, coma and death (Lidskey and Schneider, 2003).

The study carried out in Port Harcourt, Nigeria, to evaluate the degree of lead exposure and renal function tests revealed that occupationally exposed subjects had higher mean blood lead (B-Pb) 50.37 ± 24.58 µg/dL, than controls 41.40 ± 26.85 µg/dL with a significant correlation with some renal function parameters (Alasia *et al.,* 2009). This

study confirms earlier data presented by Orisakwe *et al.* (2007). The Nephrologist group of Alasia opined that level of environmental and occupational lead exposure in Port Harcourt, South-South, Nigeria is high, with occupational lead exposure increasing the risk of lead toxicity and renal function impairment. They concluded that lead exposure may be an overlooked risk factor for chronic kidney disease (CKD) in Nigeria and advocated that more studies be done and efforts be made to address the problem of lead exposure in Nigeria with the integration of these measures into preventive programs for CKD in Nigeria. Among the preventive and intervention measures cited by Alebiosu and Ayodele (2005) for the control of renal diseases, is the reduction in the exposure to heavy metals. This implies that the knowledge and awareness of possible role of some heavy metals in the etiogenesis of some chronic diseases is essential, and thus, heavy metal assay as diagnostic guide in patient management should be prioritized in healthcare settings.

2.5.3 Sources of heavy metals in the environment

Sources of heavy metals in the environment can be both natural/geological and anthropogenic. The natural or geological sources of heavy metals in the environment include weathering of metal-bearing rocks and volcanic eruptions. The global trends of industrialization and urbanization on Earth have led to an increase in the anthropogenic share of heavy metals in the environment (Nagajyoti *et al*., 2010). The anthropogenic sources of heavy metals in the environment include mining and industrial and agricultural activities. Heavy metals are released during mining and extraction of different elements from their respective ores. Heavy metals released to the atmosphere during mining, smelting, and other industrial processes return to the land through dry and wet deposition. Discharge of wastewaters such as industrial effluents and domestic

sewage add heavy metals to the environment. Phosphate fertilizers are important source of heavy metals in the environment (Nagajyoti *et al*., 2010). In Nigeria, mining of ore deposits, oil and gas exploration and exploitation, and agriculture, besides manufacturing, construction, and production activities are among the several commercial and artisanal extractions of natural resources, capable of influencing the disruption, dispersion, and distribution of naturally placed heavy metals and metalloid to the environment. Application of chemical fertilizers and combustion of fossil fuels also contribute to the anthropogenic input of heavy metals in the environment.

In general, phosphate fertilizers are produced from phosphate rock (PR) by acidulation. In the acidulation of single super-phosphate (SSP), sulfuric acid is used, while in acidulation of triple superphosphate (TSP), phosphoric acid is used (Dissanayake and Chandrajith, 2009). The final product contains all of the heavy metals present as constituents in the phosphate rock (Mortvedt, 1996). Heavy metals found in phosphate rock include; Zn, Cr, Mn, As, Cu, Fe, Ni and Pb (Faridullah *et al*., 2017). Commercial inorganic fertilizers, particularly phosphate fertilizers, can potentially contribute to the global transport of heavy metals (López Carnelo *et al.,* 1997). Heavy metals added to agricultural soils through inorganic fertilizers may leach into groundwater and contaminate it (Nagajyoti *et al*., 2010). Phosphate fertilizers are particularly rich in toxic heavy metals like Cd, Cr and Mn. The two main pathways for transfer of toxic heavy metals from phosphate fertilizers to the human body are shown below (Nagajyoti *et al*., 2010): (i) Phosphate rock $\rightarrow$ fertilizer $\rightarrow$ soil $\rightarrow$ plant $\rightarrow$ food $\rightarrow$ human body   (ii) Phosphate rock $\rightarrow$ fertilizer $\rightarrow$ water $\rightarrow$ human body

Combustion of fossil fuels in industries, homes, and transportation is an anthropogenic source of heavy metals. Vehicle traffic is among the major anthropogenic sources of

heavy metals such as Cr, Zn, Cd, and Pb (Ferretti*et al.,* 1995). Higher concentrations of environmentally important heavy metals have been reported in soils and plants along roads in urban and metropolitan areas. Regarding anthropogenic sources of heavy metals, emissions from petroleum products, coal combustion and other combustion processes are very important (Aguilera *et al*., 2021). During coal combustion, Cd, Pb, and Asare partially volatile, while Hg is fully volatile. Table 4.1 lists some environmentally important data for some key hazardous heavy metal of prime environmental concern.

2.5.4 Effects of heavy metals in the environment

Heavy metals are abundant in the environment and contribute largely to the sustainability and equilibrium of ecosystem processes (Aguilera *et al*., 2021). However, because of their bioaccumulation, non-degradability, and the excessive amounts in which they exist, these metals contaminate the food chain and subsequently become a source of toxicity to human beings and the entire ecological function. This is a major issue of concern within the study of environmental science and geochemistry. Although there is a global significance to the issue, it seems more immediate for the developing countries such as Nigeria, where the pressure of the teeming population escalates the exigency for human sustainability, food security, and total eradication of hunger (Nkwunonwo *et al*., 2020). Within the Nigerian context, many studies have examined this all-important issue, but most of these studies are fragmented and limited within the purview of mostly individual states and localities within the country. Aquatic foods, fruits, vegetables, and major staple food such as tubers are the major host of carcinogenic and mutagenic components of heavy metals in Nigeria (Nkwunonwo *et al*., 2020).

Heavy metals are potential health risk, especially in mining sites where they deteriorate from sulfide-rich ore bodies. Lead, cadmium, arsenic and mercury are very carcinogenic, while others are toxic (Obasi, and Akudinobi, 2020). Heavy metals are often associated with mining activities. Mining adversely affects the environment by inducing loss of biodiversity, soil erosion and contamination of surface water, groundwater and soil. It can also trigger the formation of sinkholes Hartman (Hartman, 1992). The leakage of chemicals from mining sites can also have detrimental effects on the health of the population living around the mining site. This has often led to water resources pollution which is a major challenge facing many developing countries of the world (U.S. Bureau of Mines, 1994). This is because mine wastes generate acid mine drainage (AMD) which contains high amount of sulfide bearing components and can migrate offsite (Khalil et al., 2013; Obiora *et al.,* 2018). Moreso, sulfide ores, including galena, are composed of heavy metal components especially lead, mercury, copper, manganese, zinc, nickel, cobalt, arsenic, selenium, molybdenum, chromium, silver and vanadium (Davies *et al*., 2005; El Amari *et al*., 2014). Some heavy metals are either essential nutrient (cobalt and zinc) or relatively harmless such as silver. However, others like cadmium, mercury and lead are highly poisonous. Several other sources of heavy metals have been implicated in the contamination of water, soil and air resulting in adverse health conditions.

2.5.5 Effects of heavy metals on microorganisms

Microorganisms are highly sensitive to heavy metal pollution and play an important role in the material cycling and energy flow of the ecosystem. Among environmental pollutants, heavy metals are the most studied because of their toxicity and non-biodegradability. Research has suggested that the specific type of metal contaminants in

a contaminated soil is a direct indication of the operation and several anthropogenic activities of man occurring at the site, resulting in heavy metal accumulations through emissions from the rapidly expanding industrial areas, disposal of high metal wastes, land application of fertilizers, animal manures, sewage sludge, accidental spillage of petrochemicals and atmospheric deposition (Khan *et al*., 2008). The range of contaminant concentrations and the physical and chemical forms of contaminants will also depend on activities and disposal patterns contributing to their persistence and microbial cell toxicity (Raulinaitis *et al*., 2012, Okoro *et al*., 2020). Therefore, the specific accumulation of cadmium and lead will inhibit soil enzymatic activities which will inadvertently have adverse effect on the soil, plants, and then humans (Liu *et al*., 2017) via the food chain or oral bioavailability of contaminated soils.

Obasi and Akudinobi (2019b), noted that heavy metals explained more of microbial community variation than the physicochemical properties; in particular, Cr and Mn negatively affected microbial α-diversity; heavy metals significantly affected the structure of microbial communities, Cr, Pb, and Zn showed uniformly negative associations with the relative abundance of bacteria *Nitrospirae* (*Nitrospira* and *Nitrospirales*), *Bacteroidetes* and *Verrucomicrobia*. Moreover, heavy metals affected predicted functions of microbial communities, including metabolic functions, genetic information processes, and functions related to the carbon cycle and the nitrogen cycle (Obasi and Akudinobi, 2019b).

Table 2.1: Sources and Toxicological Effects of some Heavy Metals

Heavy Metal	Major sources	Effect on human health	Permissible level (ppm)
Arsenic	Pesticides, fungicides, metal smelter	Bronchitis, dermatitis	0.02
Cadmium	Welding, electroplating, pesticide Fertilizer, Cd/Ni batteries, nuclear fission plant	Kidney damage, bronchitis, gastrointestinal disorder, bone marrow, cancer	0.03
Lead	Paints, pesticides, smoking, automobile, emission, mining, burning of coal	Liver, kidney, gastrointestinal damage, mental retardation in children	0.1
Manganese	Welding, fuel addition, ferromanganese production	inhalation or contact causes damage to central nervous system	0.26
Copper	mining, metal and electrical manufacturing, pesticides and fungicides, leather processing, and automotive brake pads	Mental disorders, Anaemia; Arthritis/rheumatoid arthritis; Hypertension,Nausea/vomiting,Hyperactivity, Schizophrenia, Insomnia,Autism,Stuttering,Postpartum psychosis,Inflammation and enlargement of liver,heart problem,Cystic fibrosis.	2.0
Chromium (Cr)	electroplating factories, leather tanneries, wood preserving, metal processing, chromium plating, alloy preparation, petroleum products, automobile parts, and textile manufacturing facilities	irregular heartbeats, sleep disturbances, headaches, mood changes, and allergic reactions, may increase kidney or liver damage	0.05

Source: Alluri *et al*., 2007

CHAPTER THREE

3.0 **MATERIALS AND METHODS**

3.1 **Description of Experimental Areas**

The sampling areas were five surrounding community to petroleum pipeline marketing company (PPMC) depot Suleja, and a control site, Niger State, Nigeria (Fig. 3.1) designated Plots 1-6. The community include Tunga Shanu (1), Dikko (2), Petroleum products depot premises (3), Dagwe (4), Tunga Koro (5) and Maje (6) (Table 3.1). Plots 1 and 5 are farthest to the petroleum products depot and are about 1500 meters away from the depot. They are Tunga Shanu and Tunga Koro communities respectively. Plot 4 and 6 are about 1000 meters each away from the depot while Plot 2 is 500 meters away and is closest to the petroleum products depot (Fig. 3.1). They are Dagwe, Maje and Dikko communities respectively. Plot 3 hosts the petroleum product depot. The control is the Federal University of Technology (FUT), Bosso Campus, Minna, Niger State, Nigeria, which has no previous history of oil pollution. The choice of control was to obtain isolates from petroleum free (unpolluted) habitat with similar soil characteristics as the plots (Table 3.1).

Niger State lies between Latitudes 80 20°N and 110 30°N and Latitude 3030°E and 70 20°E. Niger State lies in the central northern part of Nigeria. Suleja depot is located along Maje –Dikko by-pass in Suleja Local Government Area of Niger State. Suleja PPMC depot was established in 1995. There are 12 storage tanks in the depot with an average holding capacity of 11.2 million litres per tank. The last major fire incident at Suleja depot occurred on the 29th of January, 2017 which resulted in burning down of a petroleum loaded tanker. Long ques of petroleum tankers are usually observed along

Maje-Dikko Road leading to the depot (Appendix I). The Suleja depot is about three kilometres away from Abuja-Kaduna express highway. Communities around the PPMC depot have unauthorised mechanic workshops for the repair of broken-down vehicles. Leakages and used motor engine oils from these activities contaminate the environment (Appendix I).

Table 3.1: Distance of Samplings Plots to Petroleum Products Depot and their Coordinates

Plot	Distance (meters)	Community	Coordinates
1	1500	Tunga Shanu	9.250958° N, 7.160060° E
2	500	Dikko	9.253754° N, 7.164931° E
3	1500-(farthest communities)	Petroleum products depot premises	9.254262° N, 7.1668195° E
4	1000	Dagwe	9.2512229° N, 7.1687292° E
5	1500	Tunga Koro	9.2559345° N, 7.1786521° E
6	1000	Maje	9.255818° N, 7.1788452° E
Control	93,000	FUT Minna (Bosso campus)	9.655311° N, 6.5267310° E
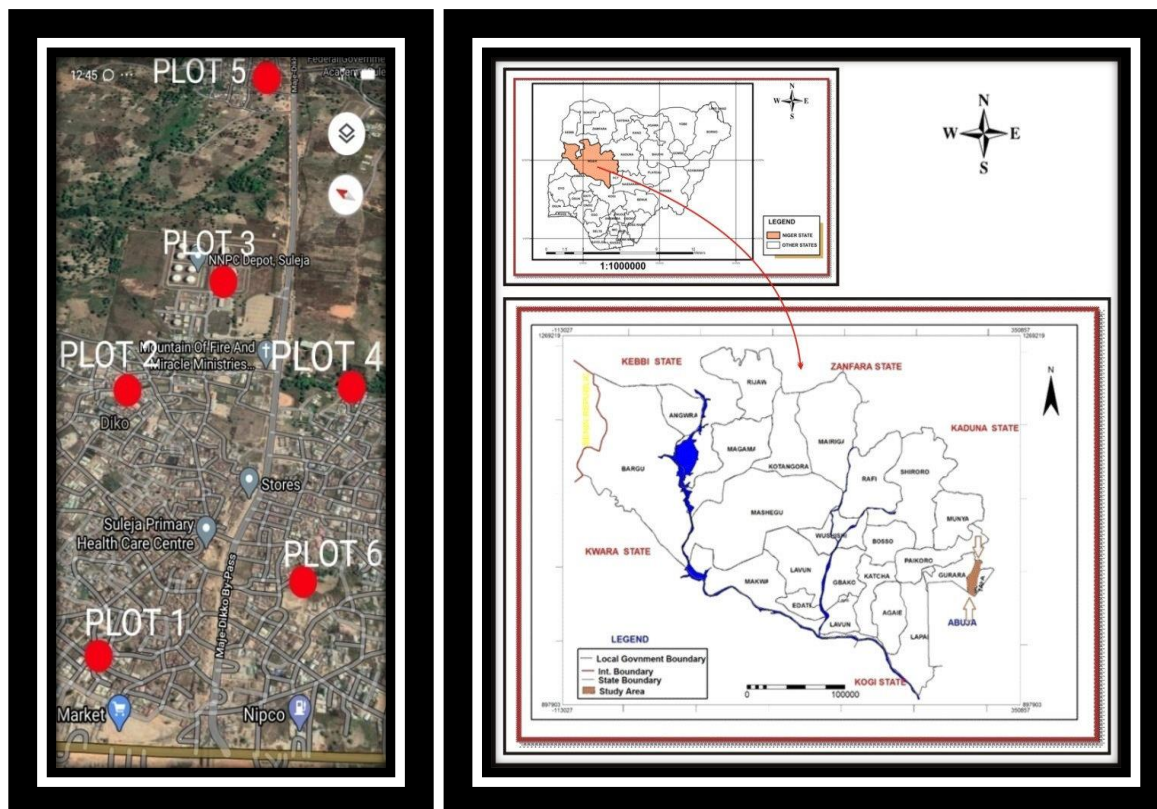
Figure 3.1: Study Site Showing the Community (Plots)

Source: Sanusi (2011)

Legend: Plot 1- Tunga shanu; Plot 2- Dikko; Plot 3- Petroleum products depot premises; Plot 4- Dagwe, Plot 5- Tunga Koro, Plot 6 Maje

3.2 Experimental Design

Complete Randomised Design (CRD) was utilised in this study for samples collection. The sample areas were divided into six plots, which include the five surrounding communities (Dikko, Maje, Tunga Shanu, Dagweru and Gwatupe) and depot premises. Five soil samples were collected from each plot while five water samples were collected from wells, boreholes and streams in each of the sub areas. In each plot, samples were collected randomly. For soil sample, ten samples were collected and bulked to make a sample. Soil and water samples were collected in both rainy (June-October) and dry (Nov.-May) seasons, for a period of 1 year.

3.3 Collection and Processing of Samples

3.3.1   Collection of soil sample

Five soil samples were collected from each of the six sub-areas. The samples were collected into nitric acid sterilized dark bottles (for PAH determination), and in clean polyethylene bags (for microbial and heavy metal analysis). Soil samples were collected with a hand auger after first stripping away litter to expose the first mineral based horizon (A-horizon). The samples were collected at depth of 0-15 cm, as described by Minnesota Pollution Control Agency, MCPA (2008). Soil samples were also collected from a community which harbour no petroleum depot to serve as control. Samples were placed in Whirlpak bags and transported to the Microbiology laboratory, Federal University of Technology (FUT), Minna, Nigeria.

3.3.2   Collection of water samples

Water samples were collected from each of the sub-areas. A total of thirty water samples were collected in sterile containers from underground wells (15 samples), boreholes (10 samples) and stream (5 samples). Samples for PAH determination were collected in dark glass bottles after rinsing the bottle with the sample and were stored at $4^{\circ}C$ with addition of 0.05 % Sodium azide (NaN_3) to inhibit microbial interferences (MCPA, 2008).Water samples were collected during rainy and dry seasons for one year. For heavy metal analysis, 3 mL concentrated nitric acid were added to samples to elevate the acidic level (pH < 2) and to reduce the activities of microorganisms in the sample in order to maintain the integrity of the samples prior to analysis (MCPA, 2008). Water samples were also collected from sources in a community with no petroleum depot in dark sterile bottles which served as control. The water samples were transported in an ice pack to the laboratory for analysis.

3.3.3   Collection of hydrocarbons

Bonny light crude oil and petroleum products (diesel, engine oil and kerosene) were collected from Kaduna Refinery and Petrochemical Company (KRPC), Kaduna, Nigeria, in clean plastic bottles. The bottles were rinsed with the product to be sampled before collection. Polycyclic aromatic hydrocarbons were purchased from Sigma-Aldrich Chemical Company (Saint Louis, Missouri US); and were used as standards.

3.4   Enumeration and Isolation of Microorganisms

3.4.1   Enumeration and isolation of microorganisms in soil

Ten grammes (10 g) of soil sample were suspended in 90 mL of sterile distilled water and were serially diluted. One millilitre of the serially diluted soil suspension was dispensed on the surface of Nutrient agar (NA) and Sabouraud dextrose agar (SDA) in Petri dishes for the enumeration of total aerobic heterotrophic bacteria (TAHB) and fungi respectively. The NA plates were incubated at 30℃ for 24-48 hours while the SDA plates were incubated at room temperature (28 ±2℃) for 3-5 days (Cappuccino and Sherman, 1981). Colonies which developed on the plates were counted and recorded as colony forming units per gram of soil (cfu/g). The isolates were sub-cultured repeatedly to obtain pure cultures, which were maintained on agar slants for further characterization and identification.

3.4.2   Enumeration and isolation of bacteria in water samples

Zero point one millilitre (0.1 mL) aliquot from the dilutions of each sample were transferred aseptically into freshly prepared Nutrient agar (NA), MacConckey agar (MCA), *Pseudomonas* base agar (PBA), thiosulfate-citrate-bile salt (TCBS) agar, Salmonella Shigella agar (SSA) and spread evenly for the isolation of TAHB, total coliform bacteria (TCB), *Pseudomonas, Vibrio, Salmonella* and *Shigella* respectively.

The inoculate plates were incubated at 37℃ for 24 -48 hours after which colonies were counted. Pure cultures of bacteria were obtained by repeatedly streaking representative colonies of different morphological types on to freshly prepared nutrient agar plates. The agar plates were incubated at 37℃ for 24 - 48 hours. Discrete bacterial colonies, which developed were characterized and identified.

3.4.3 Enumeration of total hydrocarbon utilizing bacteria and Fungi (THUB/THUF)

Total hydrocarbon utilizing bacteria (THUB) were enumerated using the method described by Hamamura *et al*. (2006) which involved the dilution of sample suspensions and plating out 0.1 mL on Bushnell Haas agar (Sigma-Aldrich, USA). Supply of hydrocarbon was carried out through the vapour phase to putative hydrocarbon utilizers by placing sterile Whatman filter papers (No.1) impregnated with 5 ml of hydrocarbon (crude oil, diesel, kerosene, engine oil) on the lids of the inverted plates. The plates were incubated at 30°C for 14 days. Colonies were counted and discreet colonies of different THUB were sub cultured on nutrient agar plates and incubated at 30°C for 24-48 hours while THUF were sub cultured on SDA plates and incubated at 28°C for 3-5days. Individual colonies were characterised and identified.

3.5   Characterization and Identification of Microbial Isolates

3.5.1   Biochemical characterization of bacterial isolates

Bacteria isolated were characterized and identified on the basis of Gram's reaction and biochemical tests as described by Cheesbrough (2003). The following biochemical tests were carried out to identify the bacterial isolates: indole, sugar fermentation, catalase, coagulase, citrate utilization, motility, spore formation, nitrate reduction, starch

hydrolysis, urease and oxidase tests. The identities of the bacterial isolates were confirmed using Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

3.5.2   Characterization and identification of fungal isolates

The fungal isolates were identified macroscopically and microscopically considering the following; aerial and substrate hyphae, type and shape of hyphae, spore formation, presence of specialized structures (foot cell, sporangiophores, conidiophores) and pigmentation.A small portion from each fungal isolate was placed in a drop of lactophenol cotton blue on a clean glass slide and then covered before viewing with x 10 and x 40 objective lenses of light microscope to confirm hyphae type. Fungal isolates were identified using Atlas of Clinical Fungi (deHoog, 1995).

3.5.3   Molecular identification of bacteria and fungal isolates

3.5.3.1 Extraction of DNA using Cetyl Trimethyl Ammonium Bromide (CTAB) method

Bacterial isolates were grown separately overnight in nutrient broth and were transferred to eppendorf tubes and spun down at 14,000 rpm for 2 mins. The fungal isolates were grown in SDA broth for 48 hours and were transferred to eppendorf tubes and spun at 14,000 rpm for 2mins. The supernatants of the isolates were then discarded and 600 µl of 2X CTAB buffer was added to the pellet and it was incubated at 65℃for 30mins. The samples were removed from the incubator and allowed to cool to room temperature and chloroform was added to each sample and gently mixed by inversion of the tube several times. Thereafter, the samples were spun at 14,000 rpm for 15 mins and the supernatants were transferred into a new eppendorf tubes and equal volume of cold Isopropanol was added to each sample to precipitate the DNA. The samples were kept in the freezer for 1h and later spun at 14,000 rpm for 10mins and the supernatants were

discarded and the pellets were washed with 70 % ethanol. The samples were thereafter air dried for 30 mins on the bench. The pellets were re-suspended in 100 µL of sterile distilled water. The extracted DNA concentrations were measured on spectrophotometer at 260 nm and 280 nm and the genomic purity was determined (Minas *et al*., 2011).

3.5.3.2 DNA electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0 % agarose gels. Preparation of agarose gels was by dissolving and boiling 1.0 g agarose in 100 mL 0.5 X TBE buffer solutions. The gels were allowed to cool to about 45℃and 10 µL of 5mg/ml ethidium bromide was added, mixed properly and poured into an electrophoresis chamber set with the combs inserted firmly. After solidification of the gels, 3 µL of the DNA with 5 µL sterile distilled water and 2 µL of 6X loading dye were mixed together and loaded in the well created. Electrophoresis was carried out at 80V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source (Hofmann and Clokie, 2018).

3.5.3.3 PCR analysis for bacteria

PCR analysis for the bacterial isolates was run with a 16S primer. The PCR mix comprises 1 µL of 10X buffer, 0.4 µL of 50 mM $MgCl_2$, 0.5 µL of 2.5 mMdNTPs, 0.5 µL 5 mM Forward primer, 0.5 µL of 5 mM Reverse primer, 0.05 µL of 5 units/µlTaq with 2 µL of template DNA and 5.05 µL of distilled water to make-up 10 µL reaction mix. The PCR profile used was initial denaturation temperature of 94℃ for 3 mins, followed by 30 cycles of 94℃ for 60 sec, 56℃ for 60 sec, 72℃ for 120 sec and the final extension temperature of 72℃ for 5 mins and the 10℃ held for 30 minutes (Hofmann and Clokie, 2018).

3.5.3.4 PCR analysis for fungi

PCR analysis for fungal isolates was run with a universal primer (1TS1 and ITS4). The PCR mix comprises 1 µL of 10X buffer, 0.4 µL of 50 mM MgCl$_2$, 0.5 µL of 2.5 mMdNTPs, 0.5 µl 5 mM ITS1 primer, 0.5µL of 5 mM ITS4 primer, 0.05 µL of 5units/µlTaq with 2 µL of template DNA and 5.05µL of distilled water to make-up 10 µL reaction mix (Farber, 1996; Wang *et al*., 2000). The PCR profile used was initial denaturation temperature of 94℃ for 3 mins, followed by 30 cycles of 94℃ for 60 sec, 56℃ for 60 sec, 72℃ for 120 sec and the final extension temperature of 72℃ for 5 mins and the 10℃ held for 30 minutes (Hofmann and Clokie, 2018).

3.5.3.5 Purification of PCR products

The amplicons were further purified before sequencing using 2M Sodium Acetate wash techniques. One µL 2 M NaAct pH 5.2 was added to approximately 10 µL of each PCR product, followed by 20 µL absolute ethanol and the mixture was kept at -20^{0}C for 1h, spun at 10,000 rpm for 10 mins, then washed with 70 % ethanol and air dried. It was re-suspended in 5 µL sterile distilled water and kept at 4^{0}C for sequencing (Hofmann and Clokie, 2018).

3.5.3.6 PCR for sequencing

The primers used for the reaction were ITS1/ITS4 and 16S for fungi and bacteria respectively. The PCR mix used were 0.5 µL of BigDye Terminator Mix, 1 µL of 5X sequencing buffer, 1 µL of M13 forward primer with 6.5 µL Distilled water and 1µL of the PCR product making a total of 10 µL. The PCR profile for sequencing had a rapid profile, the initial Rapid thermal ramp to 96℃ for 1min followed by 25 cycles of Rapid thermal ramp to 96^{0}C for 10 seconds, Rapid thermal ramp to 50℃ for 5 seconds and

Rapid thermal ramp to 60℃ for 4 minutes, then followed by Rapid thermal ramp to 4℃ (Adzitey *et al*., 2012).

3.5.3.7 Purification of PCR sequencing products

The PCR sequence product was also purified before the sequencing running using 2M Sodium Acetate wash techniques. 1µL 2M NaAct pH 5.2 was added to 10µL of the PCR product and 20µL absolute ethanol. The mixture was kept at -20℃ for 1h, spin at 10,000rpm for 10 mins, then washed with 70% ethanol and air-dried. It was re-suspended in 5µl sterile distilled water and kept at 4℃ for sequence determination.

3.5.3.8 Preparation of sample for gene sequencer

The cocktail mix was a combination of 9µl of Hi di formamide with 1µL of purified sequence making a total of 10µL. The samples were loaded on the machine (ABI Prism 3130XL Genetic Analyzer)according to the manufactures descriptions (Rahimi and Alian, 2011; Su *et al*., 2011; Adzitey *et al*., 2012).

3.6   Utilization of Petroleum Products

3.6.1   Screening test for the utilization of petroleum products by bacterial and fungal isolates

Bacterial and fungal isolates were tested for their potential to utilize petroleum products under aerobic conditions by inoculating a calibrated loopful of 24 hour old culture of microorganism into 5mL of Bushnell Haas Broth containing 0.05 mL of petroleum products (diesel, kerosene, engine oil, crude oil). Hydrocarbon utilization was screened by turbidity, total viable counts and emulsification of oil in the medium after 21 days incubation at 30°C (Kostka *et al*., 2011).

3.6.2 Utilisation efficiencies of petroleum products by selected bacterial and fungal isolates

The capacities of selected bacterial and fungal isolates to utilize crude oil were further tested in minimal salt medium (MSM). One hundred millilitres of MSM (10g NaCl, 0.29 g KCl, 0.42 g MgSO$_4$, 0.83 g KH$_2$PO$_4$, 0.42 g NaNO$_3$, 1.25 g NaHPO$_4$, 100 ml distilled water, pH 7.2) was introduced into 250 mL conical flasks and 1.0% v/v of crude oil was introduced and then sterilized using the method described by Okpokwasili and Okorie (1988).   A 24 hours old broth culture of each selected organism was seeded into each flask and incubated in a rotatory shaker incubator (New Brunswick Scientific Incubator Shaker) at 150 rev/min and 30°C. The efficiency of utilization of crude oil was monitored at three days interval for 21 days by monitoring bacterial and fungal growth measured by viable counts on solid media (NA and SDA). The optical density was also determined at 600 nm wavelength with PG T70 U.V/VIS spectrophotometer.

3.7   Utilization of Polycyclic Aromatic Hydrocarbon by Bacterial Isolates

Hydrocarbon degraders isolated from soil sample were screened for PAH utilisation using (naphthalene, anthracene, acenaphthylene) as sole carbon and energy source in an enriched salt medium incorporated with 2grammes agar (Kastner *et al*., 1994). The salt medium contained (per liter): 2.13 g Na$_2$HPO$_4$, 1.3 g KH$_2$PO$_4$, 0.5 g NH$_4$Cl, and 0.2 g MgSO$_4$. The medium was sterilized by autoclaving at 121°C for 15 min. Thereafter, 0.2 mL acetone solution containing 0.1 % (w/v) of each PAH was aseptically introduced and uniformly spread on the agar surface of dried plates (West *et al*., 1984). The acetone was allowed to evaporate under sterile condition before inoculation with pure cultures of the isolates. The inoculated plates were covered with foil and black polyethylene bag,

and then incubated in the dark at room temperature for 14 days. Colonies that formed crystal clear zones on the PAH-coated plates were replicated onto fresh PAH-coated agar plates and incubated for 14 days. Isolates that grew on these plates were selected as PAH degraders.

3.8   Determination of Total Petroleum Hydrocarbon (TPH)

3.8.1   Extraction of TPH in soil sample

Ten grammes (10 g) of soil sample were added into an amber glass bottle. Anhydrous sodium sulphate (Na_2SO_4) was equally added into the glass bottle to remove moisture from the soil sample. The sample was stirred. Three hundred microgram per millilitres (300µg/mL) of surrogate (1-chlorooctadecane) standard and 30 mL of dichloromethane (DCM) which served as extracting solvent were added to the sample. The bottle was corked very tight and transferred to a mechanical shaker (Laboratory Analytical Works Instruction, LAWI, 2011). The sample was agitated for 5 hours at room temperature using a mechanical shaker. After agitation, the sample was allowed to settle for 1 hour and then filtered through 110 mm filter paper into a clean beaker and the filtrate was allowed to concentrate to 1 mL by evaporation overnight in a fume cupboard (LAWI, 2011).

3.8.2   Extraction of TPH in water samples

Five hundred millilitres (500 mL) of water samples was transferred into 1000 mL separating flask. Thirty microgram per millilitres (30 µg/mL) of surrogate in 1 mL of DCM was added into the flask containing sample and 20 mL of DCM was added into the flask. The flask was shaken and pressure released at intervals. The sample was allowed to stand for few minutes for layers formation in the flask. The lower layer (extract) of the sample was collected into a beaker through a filter paper. The filtrate

was concentrated to 1 mL by evaporation at room temperature overnight in a fume cupboard (LAWI, 2011).

3.8.3   Purification of extracts

Sample purification was performed using glass column. Column preparation was achieved by inserting glass cotton into the column. Silica gel was dissolved with DCM to form slurry, which was added into the column. Anhydrous Na_2SO_4 was added into the column to remove any moisture present followed by addition of pentane. After preparation of the column, the concentrated sample extract was mixed with cyclohexane in a beaker and transferred into prepared column. The sample extract was eluted using pentane as solvent and eluted sample was collected in a beaker below the column. Further elution was carried out by adding more pentane into the column. After elution the column was rinsed with DCM. The eluted sample was allowed to stand overnight at room temperature in a fume cupboard for evaporation to take place (LAWI, 2011).

3.8.4   Separation and detection of TPH

The separation and detection of TPH was carried out using Agilent 6890N Gas Chromatograph - Flame Ionization Detector (GC-FID) instrument (LAWI, 2011; Cortes *et al*., 2012). Three microliters (3 μL) of concentrated sample eluted from column was injected into GC vial. The blank DCM was injected into micro-syringe of GC to clean the syringe (3 times) before taking the sample for analysis. Then the sample was injected into the column for separation of compounds in the sample. After separation the compounds were passed through a flame ionization detector. The amount of TPH was resolved at a particular chromatogram in mg/kg for soil sample and μg/L for water sample.

3.9 Determination of Polycyclic Aromatic Hydrocarbons

3.9.1 Reagents and chemicals for determination of PAHs

The chemicals and reagents that were utilised were of analytical and chromatographic grade. These include; Methylene (Riedel-de Hae-n company), n-hexane and isooctane (Merck, Germany), methanol and acetonitrile (Carlo Erba). A standard solution of the analytes (100 mg/ml each in methylene chloride) was obtained from Hewlett Packard (USA). The standards contained the following sixteen priority PAHs: naphthalene (Nap), acenaphthylene (Acl), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo[a]anthracene (BA), chrysene (Chr), benzo[b]fluoranthene and benzo[k]fluoranthene (BF), benzo[a]pyrene (BP), indeno[1,2,3-c,d]pyrene (IP), dibenzo[a,h]anthracene (dBA), benzo[g,h,i]perylene (BPer). Working standards were prepared by dilution with isooctane. Quantitative determinations were performed by means of five perdeuterated PAHs standards (1000 mg/ml each in methylene chloride, purchased from Dr. Ehrenstorfer GmbH): d8-naphthalene, d10-acenaphthene, d10-phenanthrene, d12-crysene and d12-perylene. A certified reference material (HS-3 Marine sediment) from National Research Council of Canada was used to compare the results.

3.9.2 Preparation of soil samples

A thin layer of the soil samples was screened on a filter paper to remove fragments of plants, debris, polyethylene and stones. The screened samples were air-dried under shade. The dry soil samples were grounded into powder in a ceramic mortar and demagnetized with a magnetic rod. Samples were properly labelled and were preserved in a refrigerator at $4^{\circ}C$ according to the US-EPA recommended sampling protocols

(Unite States Environmental Protection Agency, USEPA, 1998) prior to extraction and GC-MS analysis.

3.9.3 Preparation of water samples

Five millilitres aliquot of methanol were added to 100 mL of each water sample. MFC18 - 500.0 mg (Supelco, Bellefonte, PA, USA) cartridges containing the C-18 solid phase were separately wetted and conditioned using 20 mL dichloromethane, 10 mL acetone, 20 mL methanol, and finally 20 mL ultrapure water, and then dried for 3 minutes using a 12-port SPE Visi-prep vacuum manifold (Supelco, USA). The water samples were adjusted to pH 7 with sulfuric acid (1:1), and then passed through the C-18 cartridge. The PAH were eluted with 5mL acetone and 30 mL hexane. The organic extract were concentrated in a rotary evaporator (50ºC, 60 rpm), and transferred to a glass column filled with 5 g anhydrous sodium sulfate on silanized glass wool. PAH was eluted from the column using 20 mL dichloromethane. After further concentration using the rotary evaporator, the extract was transferred to 1 mL volumetric flask, and reduced under a flow of nitrogen before analysis (US Environmental Protection Agency, EPA, 1984).

3.9.4 Extraction and analysis of PAHs

The total polycyclic aromatic hydrocarbon was extracted and analysed using the method described by Nor*et al*, (2013). Ten grammes of soil sample were dissolved in 25 ml n-hexane and acetone in the ratio 7:3 (v/v) spiked with 1 ml of PAH internal standard and shaken thoroughly for proper mixing. The extractions were carried out using sonicator (Ultrasonic bath-Elmsonic S40H) under control pressure, and over a period of 40 minutes. The equipment was allowed to cool at room temperature after extraction which

was followed by filtration using Whatman glass fibre filters and kept in 25 ml universal bottles. The samples were concentrated by use of rotary evaporator to 1 ml.

Gas Chromatographic System equipped with a dual detector (FID-ECD), dual column and TriPlus AS auto-sampler with helium carrier gas and a quadrupole Mass Spectrometer (Agilent 5975 MSD) was used. Two micro litres (2µL) of extracts was injected into the GC port set at column conditions: HP-5 crosslinked PH-ME siloxane, with length of 30 m, I.D: 0.25 mm, thickness of 1 µm with helium carrier gas set in the spitless, constant flow mode with 1.2 mL/min flow rate. Other GC and MS operating set-up were carried out as specified in the operating instruction manual. Identification and quantification of individual PAHs were based on internal calibration standard containing known concentrations of the 16 PAHs. The specificity of the 16 PAHs sought for in the samples were confirmed by the presence of transition ions (quantifier and qualifier) as were shown by their retention times which corresponded to those of their respective standards (USEPA, 1984). The amount of PAHs was resolved at a definite chromatogram in mg/kg following the priciples of GC-MS (Appendix F).

3.10   Determination of Heavy Metals

3.10.1 Preparation of water samples

The water samples were first digested with aqua regia (concentrated hydrogen chloride and nitric acid, ratio 3:1 v/v). Fifty millilitres of water samples were measured into 100 mL capacity beaker, and 10mL aqua regia was added. The solution was digested until white fumes of nitric acid had escaped using a microwave digester (Berghof MWS-2). After cooling, 5 mL of concentrated nitric acid was added and the beaker covered with water glass. Gentle heating was applied until digestion was completed and content reduced to 10mL volume. The solution was filtered and transferred to 50 mL standard

flask and diluted to mark with distilled water (USEPA, 1996). The digest was used for the determination of Cd, Cr, Pb, As, Cu and Mn with Atomic Absorption Spectrophotometer (AA 500 WIN) applying the principles of AAS (Appendix G).

3.10.2 Soil samples preparation

Soil samples were air-dried and sieved using 200 mm mesh. Approximately 1g of sieved samples was weighed into 100 mL conical flasks each and was digested using 30 mL of aqua regia (concentrated hydrogen chloride and nitric acid, ratio 3:1 v/v) on a hot plate until it reduced to 20 mL volume. Digestion continued until white fumes of nitric acid disappeared and sample was reduced to 10mL volume. Adequate care was taken to prevent samples from drying to avoid loss of metals. The digest was transferred into 50 mL standard volumetric flask and made to mark with distilled water (USEPA, 1996). It was agitated vigorously and filtered with 0.45 µm Whatman filter paper. One mL of the filtrate was pipetted into another 50 mL volumetric flask and made to mark with distilled water and was used to analyse the metal concentration.

3.10.3 Determination of heavy metal concentration

The digested water and soil samples were analysed for heavy metals; chromium, cadmium, lead, arsenic, manganese and copper. Determination of heavy metal concentrations was carried out using Atomic Absorption Spectrometry, (PG 500 model AA WIN). Standards were prepared using salts of the metals. The instrument was switched on and the relevant hollow cathode lamp and wave length were fixed for each metal. Air-acetylene flame was employed. The standard for each metal was sprayed into flame as well as the samples and the corresponding absorbance values were taken. Both standards and samples were read under the same conditions. Typical set of standard calibration curves with good linear regression and better relative standard deviations

were achieved in order to measure the concentrations. Standard reference materials, NIST SRM 1643e and NIST SRM 4354 were used to verify the validity of the measurements (USEPA, 1996).

3.10.3.1 Water and soil pollution indices of heavy metal

Assessment models for pollution were used as indicators to assess the availability and intensity of anthropogenic contaminant deposition in soil and water. The following pollution assessment models were utilized in this study: Contamination Index (CI), Pollution Load Index (PLI), and Geo-accumulation Index (Igeo).

3.10.3.2 Contamination index (CI)

This is expressed as contamination factor (CF). It was used to assess soil contamination by comparing the contaminant concentration in the samples to a background/control value (Hakanson, 1980).The CI equation defined by Lacutusu (2000), was used to derive the contamination factor.

$$CF = C_n/B_n \longrightarrow 3.1$$

Where C_n = measured metal concentration and B_n = background concentration from control site. The CI classification scheme is presented in Appendix 2.

3.10.3.3 Pollution load index (PLI)

The PLI was used to derive the generalized assessment on the level of soil contamination. The PLI was obtained using the approach described by Thomilson *et al.*, (1980) as follow;

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \ldots \ldots \times CF_n)^{1/n} \longrightarrow 3.2$$

where, CF= contamination factor; and n = number of metals. The PLI classification scheme is presented in Table 2.

3.10.3.4   Geo-accumulation index (Igeo)

The Igeo enables the assessment of contamination by comparing the present heavy metals concentrations and the original pre-industrial concentrations in the soils (Muller, 1969; Chai, (2014). It was computed by the equation:

$$Igeo = Log_2 (Cn/1.5Bn) \xrightarrow{\hspace{4cm}} 3.3$$

where, Cn= measured metal concentration and Bn= control values of that metal obtained from the control site. The constant 1.5 is introduced by Ghrefat and Yusuf (2017), to minimize the effect variations in the background concentrations which may be attributed to lithologic differences. The Igeo classification scheme is presented in Appendix2.

3.11   Determination of Gene for Degradation of PAH

3.11.1 Culturing of bacterial and fungal isolates

Bacterial and fungal isolates with high potential for PAH utilization were cultured in ZoBell broth medium (5 g L^{-1} peptone, 1 g L^{-1} yeast extract, 0.01 g L^{-1} FePO$_4$, at pH 7.6) for 36 and 72h respectively at room temperature until exponential growth was achieved.The cells were collected by centrifugation (2000 rpm for 1h) and washed twice with Minimal Salt medium, MSM ((NH_4)$_2$SO$_4$, 1 μM FeSO$_4$·7H$_2$O, 100 μL 1 M KH$_2$PO$_4$, buffer solution and 10% mixed-cyclodextrin (MCD), per liter of water, at pH 7.2). The cyclohexane was allowed to volatilize by storing it in the fume hood at room temperature for 36h. Thereafter the bacterial and fungal inocula were added to 100 mL MSM with 5 ppm phenanthrene, at a final optical density of approximately OD value

0.3 (measured spectrophotometrically at 600 nm). The cultures were incubated for 24 and 72 hours at 30°C in the dark and were aggitated using a rotary shaker at 150 rpm (Hesham *et al*., 2006).

3.11.2 Extraction of fungal and bacterial DNA

Fungal and bacterial isolates were respectively transferred to eppendorf tubes and spun down at 14,000 rpm for 2 mins, the supernatant was discarded and 600 µL of 2X CTAB buffer was added to each pellet and it was incubated at 65℃ for 30 mins and 20 mins for fungal and bacterial isolates respectively. The samples were removed from the incubator and allowed to cool to room temperature and chloroform was added, the sample was mixed by gentle inversion of the tube several times. Thereafter, the sample was spun at 14,000 rpm for 15 mins and the supernatant was transferred into new eppendorf tubes and equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1 hour and later spun at 14,000 rpm for 10 mins and the supernatant was discarded and the pellets were washed with 70 % ethanol, later the samples were air dried for 30 mins on the bench. The pellets were re-suspended in 100 µL of sterile distilled water. DNA concentrations of the samples were measured on spectrophotometer at 260 nm and 280 nm and the genomic purity was determined. The genomic purity was between 1.8 and 2.0 for all the DNA samples.

3.11.3 DNA electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0 % agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 mL 0.5 X TBE buffer solutions. The gels were allowed to cool to about 45^0C and 10 µL of 5 mg/ml ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted.

After the gel has solidified, 3μL of the DNA with 5μL sterile distilled water and 2μL of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source.

3.11.4 PCR analysis for fungi using ITS1 and ITS4 primers

PCR analysis was run with a universal primer for fungi called 1TS1 and ITS4. The PCR mix comprises 1μL of 10X buffer, 0.4 μL of 50 mM $MgCl_2$, 0.5μL of 2.5 mMdNTPs, 0.5μL 5mM ITS1 primer, 0.5μL of 5mM ITS4 primer, 0.05μL of 5units/μlTaq with 2μL of template DNA and 5.05μL of distilled water to make-up 10μL reaction mix.

The PCR profile used is initial denaturation temperature of 94℃ for 3 mins, followed by 30 cycles of 94℃ for 60 sec, 56℃ for 60 sec, 72℃ for 120 sec and the final extension temperature of 72℃ for 5 mins and the 10℃ hold 30 minutes.

3.11.4.1 PCR analysis using I6S primer

PCR analysis for bacterial isolates was run with a 16S universal primer. The PCR mix comprises 1μL of 10 X buffer, 0.4 μL of 50 mM $MgCl_2$, 0.5μL of 2.5 mMdNTPs, 0.5 μL 5 mM Forward primer, 0.5 μL of 5 mM Reverse primer, 0.05 μL of 5units/μlTaq with 2 μL of template DNA and 5.05 μL of distilled water to make-up 10 μL reaction mixtures.

The PCR profile used is initial denaturation temperature of 94^0C for 3 mins, followed by 30 cycles of 94℃ for 60 sec, 56℃ for 60sec, 72℃ for 120 sec and the final extension temperature of 72℃ for 5mins and the 10℃ hold 30 minutes.

3.11.5 Purification of PCR products

The amplicon was further purified before the sequencing using 2 M Sodium Acetate wash techniques. One microliter of 2 M NaAct pH 5.2 was added to 10 µL of the PCR product, followed by 20 µL Absolute Ethanol, kept at -20℃ for 1hour and spun at 10,000 rpm for 10 mins. It was washed with 70 % ethanol, air dried and re-suspended in 5 µL sterile distilled water and kept at 4℃ for sequencing.

3.11.6 PCR for sequencing

The primers used for the reaction were ITS1/ITS4 and 16S for fungal and bacterial isolates respectively. The PCR mix used includes 0.5 µL of BigDye Terminator Mix,1µL of 5X sequencing buffer, 1µL of M 13 forward primer with 6.5 µL Distilled water and 1µL of the PCR product making a total of 10 µL. The PCR profile for Sequencing is a Rapid profile, the initial Rapid thermal ramp to 96℃ for 1min followed by 25 cycles of Rapid thermal ramp to 96℃ for 10 seconds Rapid thermal ramp to 50℃ for 5 seconds and Rapid thermal ramp to 60℃ for 4 minutes, then followed by Rapid thermal ramp to 4℃ and hold 30 minutes.

3.11.7 Purification of PCR sequencing products

The PCR sequence product was also purified before the sequencing running using 2 M Sodium Acetate wash techniques. One microliter 2M NaAct pH 5.2 and 20 µL Absolute Ethanol were added to 10 µL of the PCR product and kept at -20^{0}C for 1h, spun at 10,000 rpm for 10 mins, then washed with 70 % Ethanol, air-dried and re-suspend in 5 µL sterile distilled water and kept at 4℃ for sequencing running.

3.11.8 Preparation of sample for gene sequencing

The Cocktail mix was combination of 9 µL of Hi di Formamide with 1 µL of purified sequence making a total of 10 µL. The samples were loaded on the machine (ABI 3130 XL) and the data in form A, C, T, and G were released.

3.11.9 PCR reaction mix for PAH primers

PCR analysis was run with each of the two PAH gene primers. The PCR mix comprises 1 µL of 10X buffer, 0.4 µL of 50 mM $MgCl_2$, 0.5 µl of 2.5mMdNTPs, 0.5µL 5mM Forward primer, 0.5 µL of 5 mM Reverse primer, 0.05 µL of 5units/µlTaq with 2 µL of template DNA and 5.05 µL of distilled water to make-up 10 µL reaction mix.

The PCR profile used is initial denaturation temperature of 94^0C for 3 mins, followed by 30 cycles of 94℃ for 60 sec, 56℃ for 60 sec, 72℃ for 120 sec and the final extension temperature of 72℃ for 5 mins and the 10℃ held 30 minutes.

Table 3.3: Primer Sets Targeting Pah-degrading, 16s rDNA and Its Region Genes for Identification of Biodegrading Genes

Primer name	Genesequences
pahE1F	TGCGGCGGGTGTNAAYGGNAT
pahE1R	CCTGAGGAATCTCGGACATYTSTGCCCARAA
pahE2F	AGCATGGGAACKYTKGGNGA
pahE2R	TTTGGCGGTVACVACYTG

Source: Hanif *et al*., (2012)

3.12 Analysis of Data

Data generated were analysed using appropriate statistical tools like t-test, chi square, student t test, and Analysis of Variance (ANOVA) to establish significant differences at

95 % level of significance between various treatments. Correlation analysis was also carried out to establish relationship between various treatments. All statistical analysis was performed using SPSS 22.0 and Biological Sciences packages software (Devore, 2004).

CHAPTER FOUR

4.0   **RESULTS AND DISCUSSION**

4.1   **Results**

4.1.1 Mean counts of total aerobic heterotrophic bacteria (TAHB) in soiland water in wet and dry seasons in the various plots

The mean counts of TAHB in soil and water are depicted in Table 4.1. Soil samples had a higher TAHB mean counts than the water samples. The results also revealed that the mean counts in wet season were higher than that of dry season. Among the plots in soil samples, Plot 1 had the highest TAHB mean counts (52.0×10^6 and 26.0×10^6 cfu/mL) in wet and dry seasons respectively.The highest TAHB mean counts in water samples (9.8×10^4 cfu/mL) were recorded in plot 3 and the lowest (3.2×10^4 cfu/mL) in plot 6. The mean counts of AHB in the plots were lower than the mean counts obtained in the control.

4.1.2 Mean counts of total coliform bacteria (TCB) in soil and water in wet and dry seasons in the various plots

The mean counts of total coliform bacteria ranged from 2.0×10^4 cfu/g to 4.01×10^4 cfu/g and $2{\cdot}03 \times 10^3$ cfu/mL – $15{\cdot}00 \times 10^3$ cfu/mL in soil and water respectively (Table 4.2). The results showed that the TBC counts in both samples were high, however the mean counts were higher in the wet season than in the dry season in both soil and water samples.

Table 4.1: Mean Counts of Total Aerobic Heterotrophic Bacteria (TAHB) in Soil and Water in Wet and Dry Seasons in Various Plots

Plots	Soil samples ($\times 10^6$ cfu/g		Water samples ($\times 10^4$ cfu/mL)	
	Wet Season	Dry Season	Wet Season	Dry Season
1	52.0	26.0	6.7	4.1
2	16.1	12.1	7.6	4.4
3	62.0	12 .0	9.8	6.2
4	26.0	49.0	8.0	6.9
5	11.0	25.1	7.0	4.2
6	54.0	13.0	6.1	3.2
CT	84.0	18.0	11.5	7.9

CT = Control, cfu/g or mL = colony forming unit per gram/mililiter.

Table 4.2: Mean Counts of Total Coliform Bacteria (TCB) in Soil and Water in Wet and Dry Seasons in the Various Plots

Plots	Soil samples ($\times 10^4$ cfu/g)		Water samples ($\times 10^3$ cfu/mL)	
	Wet Season	Dry Season	Wet Season	Dry Season
1	14.01	8.08	15.00	7.10
2	5.00	3.32	8.41	6.00
3	8.50	7.35	10.00	10.01
4	3.01	4.23	4.01	5.31
5	2.11	2.02	3.10	4.03
6	2.01	2.00	4.23	2.03
CT	1.00	0.00	0.00	0.00

CT = Control, cfu/g or mL = colony forming unit per gram/mililiter.

4.1.3 Mean counts of total aerobic heterotrophic fungi (TAHF) in soil and water in wet and dry seasons in the various plots

The mean counts of aerobic heterotrophic fungi in soil ranged from 1.00×10^6 cfu/g to 14.10×10^6 cfu/g in the various plots while the mean counts of TAHF in water ranged from 1.0×10^3 cfu/mL – 4.30×10^3 cfu/mL in the various plots. The highest AHF mean counts in wet and dry seasons were 14.10×10^6 cfu/g and 6.20×10^6 cfu/g respectively and were recorded in Plot 2. The control recorded mean values of 12.00×10^6/ 6.10×10^6 cfu/g and 4.10×10^3/2.00×10^3 cfu/mL in wet/dry seasons and soil/water samples respectively (Table 4.3). The mean counts of AHF in water samples ranged from 1.10×10^3 cfu/mL to 4.30×10^3 cfu/mL in wet and dry seasons in the various plots. The highest count was obtained in Plot 2. The mean counts of AHF recorded in control were slightly than the mean counts recorded in the plots with the exception of Plot 2.

Table 4.3: Mean Counts of Total Aerobic Heterotrophic Fungi (TAHF) in Soil and Water in Wet and Dry Seasons in the Various Plots

Plots	Soil samples ($\times 10^6$ cfu/g)		Water samples ($\times 10^3$ cfu/mL)	
	Wet Season	Dry Season	Wet Season	Dry Season
1	5.20	4.10	0.00	1.10
2	14.10	6.20	4.30	3.20
3	10.10	4.50	2.00	2.20
4	3.50	3.30	1.20	0.00
5	2.20	1.20	0.00	1.12
6	3.10	1.00	2.20	1.10
CT	12.00	6.10	4.10	2.00

CT = Control, cfu/g or mL = colony forming unit per gram/mililiter.

4.1.4   Mean counts of hydrocarbon utilising bacteria and fungi in soil

The mean counts of hydrocarbon utilising bacteria and fungi are depicted in Table 4.4. Generally, the results revealed that samples from the six plots had higher HUB counts than the control plot which was not polluted by petroleum products. The mean counts of HUB ranged from 1.28×10^6cfu/g to 4.81×10^6 cfu/g in the various plots. The mean counts of HUB were highest in Plots 3 and 6 which recorded 6.32×10^6 to 5.05×10^6cfu/g and 6.11×10^6 to 5.81×10^6 cfu/g in wet and dry seasons respectively. Plot 5 recorded lowest mean counts of HUB which had counts of 1.21×10^6cfu/g and 1.20×10^6cfu/g in the wet and dry seasons respectively.

The mean counts of HUF were highest in Plot 3 with range 3.60×10^4 cfu/g to 3.05×10^4 cfu/g in wet and dry seasons respectively. The lowest mean counts of HUF were obtained in Plot 5 (Table 4.4). Plots 1, 2 and 4 had HUF mean counts in the range 1.56×10^4 - 1.80×10^4 cfu/g, $2.65 \times 10^4 – 2.11 \times 10^4$ cfu/g and $2.00 \times 10^4 – 1.93 \times 10^4$ cfu/g respectively in wet and dry seasons respectively.

Table 4.4: Mean Counts of Hydrocarbon Utilising Bacteria and Fungi in Soil

Plots	THUB ($\times 10^6$ cfu/g		THUB ($\times 10^4$ cfu/g)	
	Wet Season	Dry Season	Wet Season	Dry Season
1	1.45	2.3	1.56	1.8
2	4.20	4.00	2.65	2.11
3	6.32	5.50	3.6	3.05
4	1.42	3.40	2.00	1.93
5	1.28	2.14	1.21	1.20
6	6.11	5.81	4.5	3.71
CT	1.23	1.24	0.10	0.30

THUB/F= Total Hydrocarbon Utilising Bacteria/Fungi, cfu/g = colony forming unit per gram, CT = Control.

4.1.5 Mean counts of hydrocarbon utilising bacteria and fungi in water

The mean counts of HUB and HUF are depicted in Table 4.5. Hydrocarbon utilising bacteria recorded highest mean counts in Plot 6 (2.58×10^6 and 2.18×10^6 cfu/mL) in wet and dry seasons respectively. The mean counts of HUB were lowest in plot 5 (Table 4.5). The HUF mean counts were highest in Plot 3 with counts of 2.0×10^4 and 1.53×10^4 cfu/mL in wet and dry seasons respectively. The mean counts of HUF in Plots 1, 2, 4 and 5 were (0.95-0.6, 1.74-1.04, 0.42-0.19 and 0.13-0.15) $\times 10^4$ cfu/mL in wet and dry seasons respectively (Table 4.5). The results obtained showed that HUB and HUF mean counts were much lower in the control samples than in the six plots. The results showed that the samples in wet season had higher mean counts than the sample sin dry season. The HUB and HUF mean counts in the plots closer to the depots were also higher than ones far from the depots.

4.1.6 Morphological and biochemical characteristics of heterotrophic aerobic bacterialisolates from Soil

The morphological and biochemical characterization of bacteria from soil samples are presented in Table 4.6.The isolates were characterised and identified to their species level. There were seven Gram-positive and seven Gram-negative bacterial isolates. The isolates were identified as; *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Pseudomonas putida, Bacillus subtilis, Bacillus thuringiensis, Bacillus licheniformis, Streptococcus feacalis, Salmonella typhi, Klebsilla pneumoniae, Proteus mirabilis, Micrococcus roseus, Enterobacter aerogenes*and *Escherichiacoli* (Table 4.6).

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Table 4.5: Mean Counts of Hydrocarbon Utilising Bacteria and Fungi in Water

Plots	THUB ($\times 10^6$ cfu/mL)		THUF ($\times 10^4$ cfu/mL)	
	Wet Season	Dry Season	Wet Season	Dry Season
1	1.00	0.50	0.95	0.60
2	1.32	0.80	1.74	1.04
3	1.50	1.00	2.00	1.53
4	0.95	0.80	0.42	0.19
5	0.28	0.14	0.13	0.15
6	2.58	2.18	1.50	0.13
CT	0.40	0.10	0.05	0.00

THUB/F= Total Hydrocarbon Utilising Bacteria/Fungi, cfu/g = colony forming unit per gram, CT =Control.

4.1.7 Morphological and biochemical characteristics of heterotrophic aerobic bacteria in water

The morphological and biochemical characterization of bacteria in water samples are presented in Table 4.7. The isolates were characterised and identified to their species level. There were four Gram-positive and six Gram-negative bacteria isolates. The isolates identified were; *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcus faecalis, Salmonella typhi, Proteus mirabilis, Micrococcus roseus, Klebsiella pneumoniae, Proteus mirabilis* and *Escherichiacoli* (Table 4.7).

4.1.8 Morphological characteristics and identification of heterogeneous fungal isolates in soil and water

The morphological characteristics of fungal isolates in soil and water from the six plots are presented in table 4.8 and 4.9 respectively. The colour and shape of aerial hyphae shape of sexual spore, shape of conidiophore or sporangiophore and appearance of spore head were observed for the identification of the fungal isolates. The results obtained revealed that six isolates were identified in soil and four isolates in water samples. These isolates are from the genera *Aspergillus, Rhizopus, Mucor, and Penicillium.* The results also revealed that *Penicillium* and *Aspergillus* genera were isolated in both soil and water samples.

Table 4.6: Morphological and Biochemical Characteristics of Heterotrophic Aerobic Bacterial Isolates from Soil

Isolates Code	Gram reaction	Coagulase	Shape	Catalase	Citrate Red.	Indole	Motility	Methyl Red	VP	Starch hydrolysi	Mannitol	Fructose	Lactose	Glucose	Sucrose	Spore formatio	Egg yolk	Blood Hemolysi	Slope	H2S	Butt	MSA	Urease	Oxidize	Bacterial isolates
B1	+	+	C	+	-	-	+	-	-	-	+	+	+	+	+	-	-	-	R	-	-	-	-	-	*Staphylococcus aureus*
B2	+	-	C	-	+	-	+	-	-	-	-	+	-	-	+	-	-	β	R	-	-	-	-	-	*Streptococcus faecalis*
B3	-	-	R	+	+	-	+	+	-	+	-	-	-	+	-	-	-	-	R	+	+	-	+	-	*Proteus mirabilis*
B4	-	-	R	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	R	-	-	-	-	+	*Pseudomonas aeruginosa*
B5	+	-	R	+	+	-	+	-	-	+	-	+	+	+	+	+	-	-	R	-	-	-	-	-	*Bacillus subtilis*
B6	-	-	R	+	-	+	+	+	-	-	+	+	+	+	+	-	-	-	Y	-	-	-	-	-	*Escherichia coli*
B7	+	-	R	+	+	-	+	-	-	+	+	-	-	+	+	+	-	-	R	-	-	-	-	-	*Bacillus thuringeinsis*
B8	-	-	R	+	+	-	-	-	+	-	+	+	+	+	+	-	-	+	R	-	-	-	+	-	*Klebsilla pneumoniae*
B9	-	-	R	+	-	-	+	+	-	-	+	+	-	+	-	-	-	-	R	+	-	-	-	-	*Salmonella typhi*
B10	+	-	C	+	-	-	+	-	+	-	-	+	+	+	+	-	-	-	R	+	-	-	+	-	*Staphylococcus epidemidis*
B11	-	-	R	+	+	-	+	-	-	-	+	-	+	-	-	-	-	A	R	-	-	-	-	+	*Pseudomonas putida*
B12	+	-	R	+	-	-	+	-	+	+	+	+	+	+	+	+	-	β	R	-	-	-	-	-	*Bacillus licheniformis*
B13	+	-	C	+	+	-	+	-	+	+	-	+	-	+	-	-	-	-	R	-	-	-	-	-	*Micrococcus roseus*
B14	-	-	R	+	+	-	+	-	+	-	+	+	+	+	+	-	-	-	Y	-	-	-	-	-	*Enterobacter aerogenes*

C = cocci, R = Rod, β = Beta, - = negative, + = positive, R = red, Y = yellow

Table 4.7: Morphological and Biochemical Characteristics of Heterotrophic Aerobic Bacteriain Water

Isolates Code	Gram reaction	Coagulase	Shape	Catalas	Citrate Red.	Indole	Motility	Methyl Red	VP	Starch hydroly	Mannit	Fructos	Lactose	Glucose	Sucrose	Spore formati	Egg	Blood Haemol	Slope	H₂S	Butt	MSA	Urease	Oxidize	Bacteria isolates
Bw1	+	+	C	+	-	-	+	-	-	-	+	+	+	+	+	-	-	-	R	-	-	-	-	-	*Staphylococcus aureus*
Bw2	+	-	C	-	+	-	+	-	-	-	-	+	-	-	+	-	-	β	R	-	-	-	-	-	*Streptococcus faecalis*
Bw3	-	-	R	+	+	-	+	+	-	+	-	-	-	+	-	-	-	-	R	+	+	-	+	-	*Proteus mirabilis*
Bw4	-	-	R	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	R	-	-	-	-	+	*Pseudomonas aeruginosa*
Bw5	+	-	R	+	+	-	+	-	-	+	-	+	+	+	+	+	-	-	R	-	-	-	-	-	*Bacillus subtilis*
Bw6	-	-	R	+	-	+	+	+	-	-	+	+	+	+	+	-	-	-	Y	-	-	-	-	-	*Escherichia coli*
Bw7	-	-	R	+	+	-	-	-	+	-	+	+	+	+	+	-	-	+	R	-	-	-	+	-	*Klebsiella pneumoniae*
Bw8	-	-	R	+	-	-	+	-	-	-	+	+	-	+	-	-	-	-	R	+	-	-	-	-	*Salmonella typhi*
Bw9	+	-	C	+	+	-	+	-	+	+	-	+	-	+	-	-	-	-	R	-	-	-	-	-	*Micrococcus roseus*
Bw10	-	-	R	+	+	-	+	-	+	-	+	+	+	+	+	-	-	-	Y	-	-	-	-	-	*Enterobacter aerogenes*

C = cocci, R = Rod, β = Beta, - = negative, + = positive, R = red, Y = yellow

Table 4.8: Morphological Characteristics and Microscopic Features of Fungal Isolates in Soil

Isolate code	Colour of aerial hyphae	Morphology of hyphae	Shape of asexual spore	Morphology of sporangiophore/ conidiophores	Appearance of spore head	Fungal species
Fs1	White grey/olive-green	Non- septate	Ovoid with truncate base	Unbranded sporangiophore	Swollen, nucleated	*Mucorplumeus*
Fs2	Black	Septate	oval	Long, erect non-septate sporangiophore	Swollen, multi-nucleated	*Aspergillusniger*
Fs3	Green	Septate	oval	Long septate conidiophore	Swollen/Multinucleated	*Penicilliumnotatum*
Fs4	White	Non- septate	Oval	Sporangiophore with colummelae	Swollen, round	*Rhizopusstolonifer*
Fs5	White	Septate	oval	Unbranded sporangiophore	Swollen, unnucleated	*Aspergillusflavus*
Fs6	Brown	Septate	oval	Long standing non-septate sporangiophore	Swollen, nucleated	*Aspergillus fumigatus*

Table 4.9: Morphological Characteristics and Microscopic Features of fungal Isolates in Water

Isolate code	Colour of aerial hyphae	Morphology of hyphae	Shape of sexual spore	Morphology of sporangiophore/ conidiophores	Appearance of spore head	Fungi specie
Fwa	White	Septate	Oval	Unbranded sporangiophore	Swollen, unnucleated	*Aspergillusflavus*
Fwb	black	Septate	Oval	Long,erect, non-septate sporangiophore	Swollen, multi-nucleated	*Aspergillusniger*
Fwc	green	Septate	Oval	Long septate conodiophore	Swollen/Multinucleated	*Penicilliumnotatum*
Fwd	White	Non- septate	Oval	Sporangiophore with colummelae	Swollen, round	*Rhizopusstolonifer*

4.1.9 Occurrence of bacterial isolates in the various plots

The bacterial isolates were not evenly distributed in the six experimental plots and control plot. The control had the least numbers of isolated bacteria. The result revealed that Plots 2 and 6 recorded 11 isolates each. Plots 2 and 6 which were closest to the PPMC depot had total coliform and hydrocarbon utilising bacteria in addition to other isolates and recorded the highest numbers of isolates (Table 4.10). Plots 4 and 5 recorded 8 and 6 isolates respectively while plot 1 had 7 isolates. Plot 3 which hosts the depot had 8 isolates which were predominantly HUB. This could be as a result of availability of hydrocarbon in the plots which attracts HUB. The results from the 6 plots revealed that *Bacillius* and *Pseudomonas* genera were predominant and were isolated in all the plots.

4.1.10 Occurrence of fungal isolates in the various plots

Table 4.11 shows the spread of fungal isolates in the various plots. The results revealed that fungi were isolated in all the plots but they were not evenly spread out. The control plot had the least numbers of fungal isolates and recorded only 2 fungi genera (Table 4.11). Plots 2 and 6 had more fungal spread than the other plots and recorded 5 and 6 fungal genera respectively.

Table 4.10: Occurrence of Bacterial Isolates in the Various Plots

Bacterial isolates	Plot 1	Plot2	Plot3	Plot4	Plot5	Plot6	Control	Occurrence (%)
Staphylococcus aureus	-	+	+	+	+	+	+	87.71
Streptococcus faecalis	+	-	-	+	-	+	-	42.86
Proteus mirabilis	-	+	-	-	-	-	-	14.29
Pseudomonas aeruginosa	+	+	+	+	+	+	+	100
Bacillus subtilis	+	+	+	+	+	+	+	100
Escherichia coli	-	+	-	+	-	+	-	42.86
Bacillus thurengiensis	+	+	+	+	+	+	+	100
Klebsilla pneumoniae	-	+	-	-	-	-	-	14.29
Salmonella typhi	-	+	-	-	-	+	-	28.60
Staphylococcus epidemidis	+	-	+	+	-	+	-	57.14
Pseudomonas putida	+	+	+	-	+	+	-	71.43
Bacillus licheniformis	+	+	+	+	+	+	+	100
Micrococcus roseus	-	-	-	-	-	-	+	14.29
Enterobacter aerogenes	-	+	-	-	-	+	--	28.57

Key: + = positive, - = absent

Table 4.11: Occurrences of Fungal Isolates in the Various Plots

Fungal isolates	Plot 1	Plot2	Plot3	Plot4	Plot5	Plot6	Ctr	Occurrence (%)
Penicillium notatum	+	+	+	+	+	+	+	100.00
Aspergillus niger	+	+	+	-	+	+	-	71.43
Rhizopus stolonifer	-	+	-	-	-	+	-	28.57
Aspergillus flavus	-	+	+	+	-	+	-	57.14
Mucor plumbeus	+	-	-	-	-	+	+	42.86
Aspergillus fumigatus	-	+	-	-	+	+	-	42.86

Key: + = positive, - = absent, Ctr = control

4.1.11 Utilization of petroleum products by bacterial isolates

The ability of selected bacterial isolates from the various plots with potential to degrade hydrocarbons were tested on some petroleum products (Crude oil, kerosene, Diesel and engine oil), which severed as sole source of carbon and energy in modified minimal salt broth. The bacterial isolates utilized the petroleum products to varying capacities after 21 days of incubation (Table 4.12).

Crude oil had the highest absorbance of 36.04 % at 600 nm while the engine oil had the lowest absorbance of 15.81 % at 600 nm. The percentage absorbance of Kerosene and Diesel by the bacterial isolates was 28 % and 19.60 % respectively. Bacterial isolates from the genera *Pseudomonas* and *Bacillus* had the highest absorbance of 0.663±0.55 and 0.601±0.14 respectively on crude oil. The least absorbance on crude oil (0.081±0.41 nm) was from *Proteus mirabilis. Streptococcus faecalis* and *Proteus mirabilis* among the hydrocarbon utilising bacteria were least efficient in the utilisation of the petroleum products.

4.1.12 Utilization of petroleum products by fungal isolates

The ability of isolated fungi to utilise some petroleum products (Crude oil, Kerosene, Diesel, and Engine oil) as sole source of carbon and energy was determined in modified minimal salt broth. The fungi utilised the petroleum products to varying capacities within 21 days of incubation (Table 4.13). The highest percentage utilisation was in crude oil (43.16 %) while the lowest was in engine oil (12.64 %). Kerosene and Diesel recorded percent utilisation of 25.66 % and 18.71 % respectively by the fungal isolates. Among the fungal species, *Aspergillus niger* and *Penicillium notatum* exhibited greater capacity to utilise the petroleum products when compared with other fungal isolates (Table 4.13).

Table 4.12: Utilization Efficiencies of Petroleum Products by Bacterial Isolates after 21 Days of Incubation

Bacterial Isolates	Crude oil	Kerosene	Diesel	Engine oil	Total (%)
Staphylococcus aureus	0·302±0.21	0·291±0.31	0·250±0.13	0·180±0.15	1·023 (11·10)
Bacillus subtilis	0·580±0.13	0·391±0.13	0·291±0.25	0·264±0.26	1·526 (16·56)
Bacillus thuringensis	0·601±0.15	0·413±0.42	0·311±0.46	0·284±0.24	1·609 (17·46)
Streptococcus faecalis	0·131±0.34	0·049±0.38	0·030±0.59	0·062±0.33	0·317 (3·44)
Pseudomonas aeruginosa	0·663±0.55	0·431±0.02	0·281±0.23	0·250±0.16	1·625 (17.60)
Proteus mirabilis	0·081±0.41	0·103±0.35	0·125±0.31	0·031±0.11	0·340 (3·69)
Staphylococcus epidemidis	0·180±0.30	0·116±0.41	0·135±0.21	0·051±0.42	0·482 (5·23)
Bacillus licheniformis	0·282±0.40	0·381±0.16	0·190±0.29	0·152±0.13	1·005 (10·91)
Pseudomonas putida	0·501±0.25	0·410±0.44	0·193±0.18	0·183±0.43	1·287 (13·93)
Total	3·321 (36·04)	2·630 (28.54)	1·806(19·60)	1·457(15·81)	9·214 (100)

Values are replicate mean determinations ± SD

Table 4.13: Utilization Efficiencies of Petroleum Products by Fungal Isolates after 21 Days of Incubation

Fungal Isolates	Crude oil	Kerosene	Diesel	Engine oil	Total
Aspergillus niger	0·713±0·41	0.453±0·31	0.325±0·43	0.213±0·14	1.704(24.1)
Penicillium notatum	0.654±0·10	0.412±0·25	0.250±0·15	0.213±0·23	1.529(21.23)
Mucor plumeus	0.301±0·2	0.125±0·11	0.181±0·22	0.051±0·15	0.658(9.31)
Rhizopus stolinifer	0.211±0·41	0.124±0·23	0.053±0·33	0.024±0·43	1.494(21.13)
Aspergillus fumigatus	0.648±0·30	0.386±0·17	0.260±0·32	0.200±0·41	0.42(5.941)
Aspergillus flavus	0.524±0·15	0.314±0·28	0.254±0·25	0.180±0·18	1.272(17.99)
Total	3.051(43.16)	1.814(25.66)	1.323(18.71)	0.881(12.46)	7.069(100)

Values are replicate mean determinations ± SD

4.1.13 Meanviable cell counts of selected hydrocarbon utilising bacteria and fungi in MSM amended with crude oil

There were variations in the viable cell counts of selected HUB and HUF in minimal salt broth amended with crude oil during 21 days of incubation. The crude oil in the medium gradually emulsified as the degradation time progressed. There were increases in the viable cell counts up to day 15 and 18 for *Bacillus thuringiensis* and *Pseudomonas aeruginosa* species respectively. *Pseudomonas aeruginosa* recorded the highest viable cell counts when compared with other isolates (Table 4.14). The viable cell counts increased from 4.32×10^6 to 12.15×10^6 cfu/mL and declined to 12.00×10^6 cfu/mL on day 21. *Penicillium* and *Aspergillus* recorded a consistent increase in viable counts to 5.80×10^4 and 7.60×10^4 up in day 15 and 18 respectively.

Table 4.14: Mean Viable Cell Counts of Selected Hydrocarbon Utilising Bacteria and Fungi in MSM amended with Crude Oil

| Time(days)	*Bacillus thuringiensis*	*Pseudomonas aeruginosa*	*Penicillium notatum*	*Aspergillus niger*
	VCC($\times 10^6$)		VCC($\times 10^4$)	
0	3.40	4.32	2.60	3.30
3	3.80	4.71	3.70	4.11
6	4.61	5.70	4.50	5.23
9	4.91	6.15	4.90	6.30
12	5.82	8.45	5.60	5.51
15	7.21	10.71	5.80	7.40
18	6.50	12.15	5.00	7.00
21	4.51	12.00	4.81	5.20

VCC = viable cell count

4.1.14 Molecular identification of screened bacterial and fungal isolated used for PAH degradation

Two bacterial and fungal isolates each with the highest PAH degrading capacities were identified using molecular analysis. The bacteria isolates (B7 and B4) analyzed had 98.8 % and 99.0 % similarity to *Bacillus thuringiensis* strain M43 and *Pseudomonas aeruginosa* strain PA14 respectively after characterization of 16S ribosomal RNA gene. The DNA images of *Bacillus thuringiensis* strain M43 and *Pseudomonas aeruginosa* strain PA14 amplified by 16s RNA at 1600BP by Gel electrophoresis were presented in Plate I. The gene sequences of *Bacillus thuringiensis* strain M43 and *Pseudomonas aeruginosa* strain PA14 were presented in Appendix D. The fungal isolates (Fs2 and Fs3) analyzed had 98.5 % and 98.9 % similarity to DNA images of *Aspergillus niger* strain ATCC 1015 and *Penicillium notatum* strain P72-30 amplified by 16s RNA at 500 BP by Gel electrophoresis (Plate II). The gene sequences of *Aspergillus niger* strain ATCC 1015 and *Penicillium notatum* strain P72-30 were presented in Appendix D.
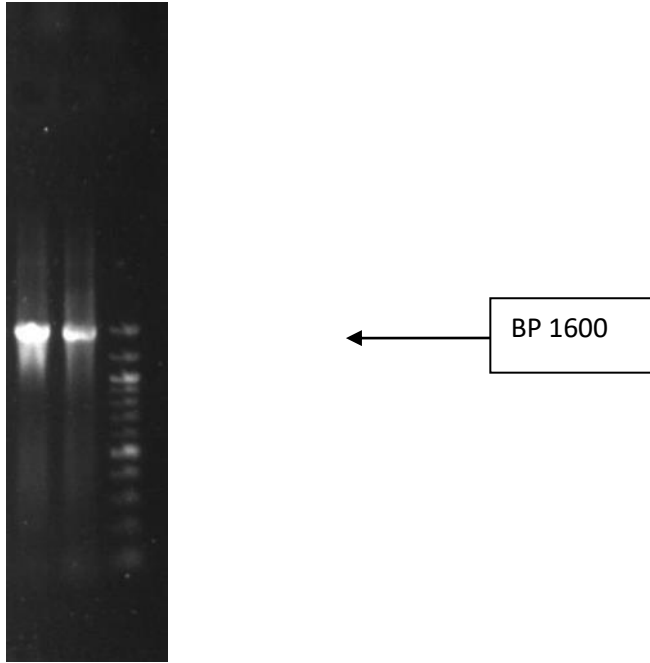
Plate I: DNA images of *Bacillus thuringiensis* strain M43 and *Pseudomonas aeruginosa*strain PA14 amplified by 16s RNA at 1600BP by Gelelectrophoresis
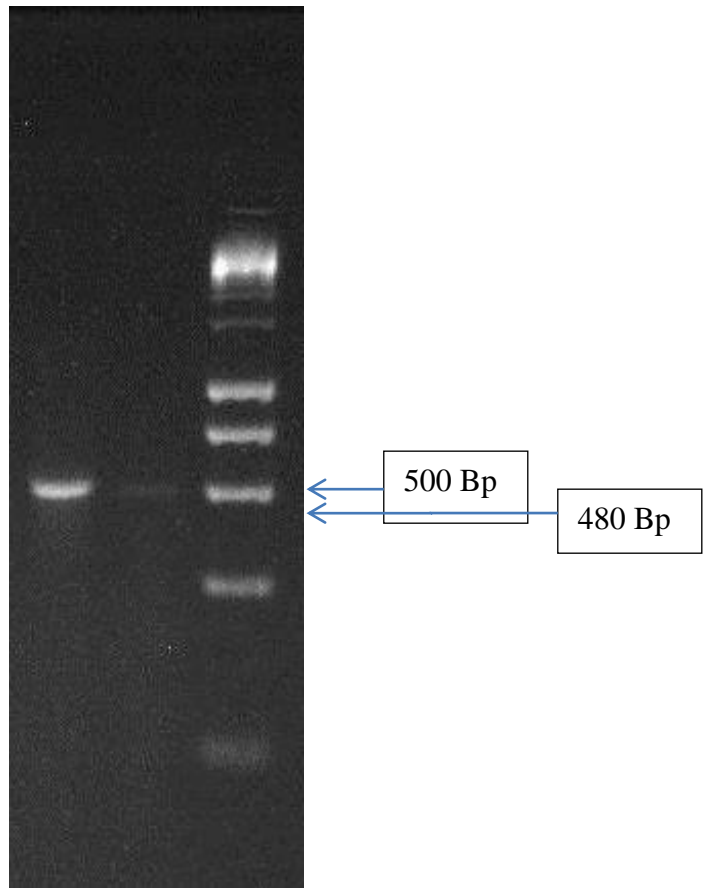
Plate II: DNA images of *Aspergillus niger* strain ATCC 1015 and *Penicillium notatum* strain P72-30 amplified by 16s RNA at 500Bp by Gelelectrophoresis

4.1.15: Heavy Metals in Soil during Rainy Season

The concentrations (mg/kg) of heavy metals (Cr, Cd, Pb, As, and Cu) in soil during rainy season in the various plots are presented in Table 4.15. Plot 6 had the highest concentration of Cr, Cd, Pb, As and Cu, while the control had the highest concentration of Mn. The least concentration of Cr, Cd and Cu was recorded in Plot 4 while Plot 5 had the least concentration of Pb and Mn. There were significant differences in the concentration of the heavy metals in the various plots.

4.1.16: Heavy Metals in Soil during Dry Season

The concentrations of heavy metals in soil during dry season in the various plots are shown in Table 4.16. The highest concentration of Cr was recorded in Plot 2, the highest concentration of Cd and Pb was recorded in Plot 1. Plot 5 had the highest concentration of As, Plot 6 had the highest concentration of Mn and the control had the highest concentration of Cu (Table 4.16).

Table 4.15: Mean Concentration of Heavy Metals in Soil During Rainy Season in the Various Plots

Plots	Heavy metals (mg/kg)					
	Cr	Cd	Pb	As	Mn	Cu
1	0.40±0.02 [b]	2.38±0.06 [b]	6.76±0.46[ab]	2.35±0.05[a]	3.99±0.32[a]	1.50±b0.45[a]
2	0.68±0.17 [b]	1.76±0.03 [ab]	7.37±0.73 [bc]	2.50±0.10[ab]	2.96±0.27[a]	2.46±0.41 [a]
3	0.65±0.06 [dc]	2.20±0.58 [b]	6.09±0.41 [bc]	1.42±0.49[ab]	3.38±0.13[a]	3.32±0.05 [c]
4	0.35±0.53 [bc]	1.69±0.55 [ab]	5.60±0.12 [c]	1.96±0.12 [a]	2.78±0.12[a]	1.23±0.58 [bc]
5	0.52±0.40 [a]	2.56±0.84 [a]	5.12±1.10 [a]	2.42±1.10 [a]	2.56±0.98[a]	2.04±0.01 [ab]
6	1.21±0.00 [d]	3.15±0.00 [c]	8.27±0.04 [d]	2.58±0.40 [b]	2.99±1.47[a]	2.49±0.04 [c]
CT	0.10±0.04 [bc]	0.01±0.3 [ab]	0.01±0.47 [abc]	0.47±0.16 [a]	5.04±0.13[a]	1.19±0.03 [bc]

Values are x̄±SEM of duplicate values x̄ with dissimilar letter(s) are not significantly different (P>0.05) from each other according to Duncan Multiple Range Test (DMRT).

Table 4.16: Mean Concentration of Heavy Metals in Soil During Dry Season

Plots	Heavy Metals (mg/kg)					
	Cr	Cd	Pb	As	Mn	Cu
1	0.38±0.03 a	2.76±0.08 c	6.48±0.67 ab	2.01±0.25 ab	2.84±0.07 ab	1.54±0.03 ab
2	0.68±0.05 b	2.06±0.06 bc	5.57±1.42 ab	2.17±0.23 bc	3.08±0.01 b	1.85±0.32 ab
3	0.43±0.25 c	2.12±0.25 bc	6.27±0.11 ab	1.43±0.39 ab	2.15±0.15 b	1.33±0.63 ab
4	0.28±0.40 a	2.12±0.03 ab	7.61±0.49 a	2.42±0.28 a	2.04±0.35 ab	2.42±0.06 a
5	0.60±0.02 c	2.67±0.41 ab	4.11±0.93 b	3.23±0.79 bc	3.36±0.28 b	1.88±0.08 ab
6	0.26±0.04 d	2.35±0.32 d	4.20±1.27 b	0.47±0.03 ab	4.75±0.68 ab	1.10±0.58 ab
CT	0.07±0.30 bc	0.17±0.00 a	0.00±0.00 c	1.70±0.04 c	1.40±1.39 a	2.54±0.00 a

Values are x̄± SEM of duplicate values. x̄ with dissimilar letter(s) are not significantly different (P>0.05)
from each other according to Duncan Multiple Range Test (DMRT).

4.1.17 Heavy metals in water during rainy season

The concentrations of heavy metals in water during rainy season in the various plots are shown in Table 4.17. The highest concentration of Cr and Cd was recorded in Plot 4, while the highest concentrations of Pb, As, Mn, and Cu were recorded in Plots 1, 3, 6, and 5 respectively. The highest concentration of Cu was recorded in Plot 5. The control had the lowest concentration of all heavy metals with the exception of Mn which was higher than all other plots (Table 4.17).

4.1.18 Heavy metals in water during dry season

The concentrations of heavy metals (Cr, Cd, Pb, As, Mn, and Cu) in water during dry season in the various plots are presented in Table 4.18. The highest concentrations of Cr, Cd, and Cu were observed in Plot 1. The least concentrations of Cr, Cd, Pb, As, and Cu were observed ND in Plot 2. However, Plot 2 had the highest concentration of Mn. Plots 3 and 5 had the highest concentrations of Pb and As respectively (Table 4.18).

Table 4.17: Mean Concentration of Heavy Metals in Water during Rainy Season

Plots	Heavy Metals (mL/l)					
	Cr	**Cd**	**Pb**	**As**	**Mn**	**Cu**
1	0.2±0.066[bdc]	0.23±0.06[de]	3.47±0.06[a]	1.23±0.04[c]	2.07±0.4[bc]	0.52±0.03[e]
2	0.46±0.05[ab]	1.14±0.08[ab]	2.42±0.20[b]	1.60±0.05[b]	2.12±0.70[bc]	1.08±0.03[b]
3	0.53±0.10[a]	0.85±0.23[bc]	2.47±0.12[b]	2.24±0.09[a]	1.47±0.28[d]	0.85±0.06[c]
4	0.63±0.06[a]	1.25±0.07[a]	3.31±0.01[a]	1.79±0.22[b]	1.73±0.08[cd]	0.67±0.02[d]
5	0.42±0.11[abc]	0.52±0.02[cd]	1.41±0.04[a]	0.92±0.03[c]	1.94±0.02[bcd]	1.26±0.04[a]
6	0.20±0.02[cd]	1.16±0.02[ab]	1.69±0.06[c]	0.58±0.04[d]	2.35±0.05[ba]	1.16±0.02[ab]
CT	0.05±0.02[d]	0.01±0.00[e]	0.02±0.00[d]	0.00±0.00[e]	2.80±0.20[a]	0.58±0.06[d]

Values are x̄±SEM of duplicate values. x̄ with dissimilar letter(s) are not significantly different(P>0.05) from each other according to Duncan Multiple Range Test (DMRT).

Table 4.18: Mean Concentration of Heavy Metals in Water during Dry Season

Plots	Cr	Cd	Pb	As	Mn	Cu
1	1.24±1.00 [a]	1.95±0.19 [a]	2.37±0.31 [bc]	0.31±0.21 [e]	2.53±0.22 [c]	1.15±0.13 [a]
2	0.05±1.00 [e]	0.01±0.00 [f]	0.01±0.00 [d]	0.00±0.00 [f]	3.75±0.25 [a]	0.00±0.00 [e]
3	0.20±0.11 [de]	0.39±0.06 [e]	3.72±0.04 [a]	1.10±0.05 [c]	2.59±0.05 [c]	0.04±0.01 [c]
4	0.59±0.04 [b]	1.01±0.05 [c]	3.29±0.17 [ab]	1.33±0.03 [b]	3.06±0.04 [b]	0.95±0.06 [b]
5	0.43±0.07 [bc]	1.92±0.03 [a]	1.99±1.00 [d]	2.16±0.07 [a]	1.14±0.05 [e]	0.64±0.03 [c]
6	0.58±0.01 [b]	1.21±0.35 [b]	2.67±0.03 [bc]	2.08±0.07 [a]	1.90±0.03 [d]	0.45±0.06 [d]
CT	0.29±0.07 [dc]	0.64±0.02 [d]	2.20±0.13 [e]	0.79±0.00 [d]	2.02±.09 [d]	1.13±o.09 [a]

Values are x̄±SEM of duplicate values. x̄ with dissimilar letter(s) are not significantly different ($P > 0.05$)
from each other according to Duncan Multiple Range Test (DMRT).

4.1.19 Geo- accumulation indices (Igeo) of heavy metals in soil

The Geo- accumulation indices of the soil showed the degree of anthropogenic pollution of the soil in both rainy and dry seasons. The Igeo ranged from slightly polluted to moderately – severely polluted in the various plots. In Plot 1, Cd, Cr, and As were responsible for high Igeo and were categorized as moderately polluted whereas, Pb with Igeo value of 2.44 was reported as severely polluted. Plots 4 and 6 had the lowest Igeo in manganese, 0.9 and 0.8 respectively. In all the samples Mn was relatively recorded unpolluted – slightly polluted in the various plots and seasons. Plot 3 had Igeo Pb 2.34 (severely polluted) and was moderately polluted with As, Cr, and Cd (Table 4.19). The mean Igeo for Cr, Cd, Pb, As, Mn and Cu were 1.45, 1.42, 2.32, 1.60, 0.27 and 0.34 respectively.

4.1.20 Geo- accumulation Indices (Igeo) of heavy metals in water

The geo-accumulation index revealed that water samples from Plot1 were severely polluted with As, slightly polluted with Cd, Pb, Mn and Cu and were moderately polluted with Cr. Samples from the petroleum products depot (Plot 3) were slightly polluted with Cr, Cd, Cu, Mn, and Pb but were severely polluted with As. Plot 6 was slightly polluted with Cu, Mn and Pb and moderately polluted with Cr and Cd, but severely polluted with As. With the exception of Plots 5 and 2 which were moderately polluted and moderately to severely polluted with As respectively, other plots were severely polluted with As. The metals responsible for the rise in geo-accumulation indices are in this decreasing order; As (2.99) > Cr (1.07) > Cd (0.88) > Pb (0.63) >Cu (0.29) > Mn (0.14) (Table 4.20).

Table 4.19: Geo- accumulation Indices of Heavy Metals in Soil Samples

Plots	Igeo-Cr	Igeo-Cd	Igeo -Pb	Igeo- As	Igeo- Mn	Igeo -Cu	Total
1	1.37	1.61	2.44	1.62	0.13	0.26	7.43
2	1.49	0.99	2.41	1.72	1.12	0.36	8.09
3	1.46	1.34	2.34	1.00	0.11	0.39	6.64
4	1.39	1.19	2.41	1.63	0.09	0.30	7.01
5	1.45	1.65	1.92	2.00	0.11	0.33	7.46
6	1.56	1.76	2.39	1.60	0.08	0.42	7.81
Mean- Igeo	1.45	1.42	2.32	1.60	0.27	0.34	7.41

Table 4.20: Geo- accumulation Indices of Heavy Metals in Water Samples

Plots	Igeo-Cr	Igeo-Cd	Igeo -Pb	Igeo- As	Igeo- Mn	Igeo –Cu	Total
1	1.05	0.24	0.89	3.94	0.16	0.14	6.42
2	1.00	0.85	0.70	2.90	0.18	0.35	5.98
3	0.86	0.11	0.55	3.94	0.09	0.26	5.36
4	1.07	0.98	0.71	3.31	0.12	0.19	6.38
5	0.99	1.85	0.45	1.13	0.14	0.41	4.97
6	1.49	1.24	0.50	3.19	0.17	0.39	6.98
Mean-Igeo	1.07	0.88	0.63	2.99	0.14	0.29	6.02

4.1.21 Contamination factor and pollution load indices (PLI) in soil

Analysis of the contamination factors showed that plots were moderately contaminated to slightly polluted with Cu and Mn, but severely polluted with Cr, Pb, As and Cd. Plots 1 and 6 were very severely polluted with Pb and Cd respectively (Table 4.21). The contamination factors of the metals are in this order; Pb, > Cd > Cr > As > Cu > Mn. The highest contamination factor was recorded on cadmium in Plot 6 (Table4.21).

4.1.22 Contamination factor and pollution load indices (PLI) in water

The contamination factor showed severe contamination to slight pollution of Cu and Mn across the entire plots, slight pollution to severe pollution of Cd, severe pollution of Cr, and very severe pollution of Pb in the entire plots. The PLI showed that the pollution ranged from highly polluted to very highly polluted levels with the heavy metals. Plot 1 had the lowest pollution index (4.05) while Plots 3 and 6 recorded PLI of 4.90 and 5.60 respectively. The pollution load indices of the plots strongly suggested that the water samples were polluted with the investigated heavy metals to various degrees.

Table 4.21: Contamination Factor and Pollution Load Indices in Soil Samples

Plots	Cr C_f^i	Cd C_f^i	Pb C_f^i	As C_f^i	Mn C_f^i	Cu C_f^i	C_f^i total	PLI
1	6.84	8.03	8.17	4.62	0.67	1.28	29.61	3.48
2	7.47	4.96	7.98	4.96	0.59	1.81	27.77	3.41
3	7.26	6.68	7.62	3.02	0.55	1.95	30.70	3.26
4	6.90	5.93	8.14	4.64	0.47	1.31	27.40	3.00
5	7.15	8.22	5.69	6.00	0.58	1.65	29.29	3.53
6	7.80	8.78	7.55	4.55	0.43	2.11	31.22	3.60
Total	43.42	42.60	45.15	22.79	3.29	10.33	172.58	20.28
C_f^i mean	7.24	7.10	7.53	4.63	0.55	1.72	28.76	3.38

Table 4.22: Contamination Factor and Pollution Load Indices in Water Samples

Plot	Cr C_f^i	Cd C_f^i	Pb C_f^i	As C_f^i	Mn C_f^i	Cu C_f^i	C_f^i total	PLI
1	5.55	1.24	32.72	33.33	0.83	0.71	74.38	4.05
2	5.33	4.28	26.00	16.22	0.93	1.74	54.5	5.06
3	4.55	5.52	20.27	24.33	0.82	1.28	56.77	4.90
4	5.66	4.92	27.09	21.44	0.64	0.96	60.71	4.62
5	7.88	9.24	16.36	4.77	0.71	2.05	41.01	4.49
6	7.88	6.20	18.45	19.77	0.87	1.98	55.15	5.60
Total	36.85	31.4	140.89	119.86	4.80	8.72	342.52	28.72
C_f^i mean	6.14	5.23	23.48	19.98	0.8	1.45	57.08	4.78

4.1.23 Total petroleum hydrocarbon concentrations in soil in the various plots.

The results of the TPHs obtained from Plots 1-6 including the control in the soil samples were presented in Tables 4.23 - 4.29 respectively. The results obtained were 1407.18 mg/kg, 3153.25 mg/kg, 4808.59 mg/kg, 2593.36 mg/kg, 2879.27 mg/kg and 4137.98 mg/kg for Plots 1-6 respectively. The mean concentration of total petroleum hydrocarbon in the plots was 3173.77 mg/kg. The concentration in the control site was slightly higher than 25.82 mg/kg. Plot 3 recorded the highest concentration of TPH (4808.59 mg/kg) followed by Plots 6 (4137.98 mg/kg), Plot 2 (3153.25 mg/kg), Plot 5 (2879.27 mg/kg), Plot 4 (2593.36 mg/kg) and Plot 1 (1407.18 mg/kg) which had the lowest concentration.

The TPH results of TPH in the various plots varied for the hydrocarbon peak numbers. Plot 1 recorded highest concentration in C40 (529.7107 ppm) and lowest in C33 (2.4444 ppm) (Table 4.23). Total petroleum hydrocarbon was highest in C9 (1840.7954 ppm) followed by C8 and C40 (448.7936 and 244.3564 ppm) respectively and lowest in C24 (2.3437) in Plot 2 (Table 4.24). Plot 3 recorded highest concentration inC10 (1617.5858 ppm) and lowest in concentration in C24 (0.6170 ppm) (Table 4.25). Plot 4 had highest concentration of 1187.8043 ppm (C10) and lowest concentration of 2.3435 ppm (C38) (Table 4.26). Plots 5 and 6 recorded highest concentrations of TPH in C9 (902.9778 ppm) and C9 (1985.1532 ppm) respectively while the lowest concentrations were in C28 (1.6416 ppm) and C35 (4.5905 ppm) respectively (Tables 4.27 and 4.28).

4.1.2   Total petroleum hydrocarbon concentrations in water in the various plots

The concentration of total petroleum hydrocarbons obtained from the water samples from the plots including the control are depicted in Table 4. 30 – 4.36. The results ranged from 140.10 mg/L – 352.00 µg/L in the entire plots. Total mean concentration of 223.50 was obtained from the plots. The highest concentration was observed in Plot 3 (352.01 µg/L) followed by Plot 6 (325.22 µg/L), Plot 1 (191.20 µg/L), Plot 2 (188.29 µg/L), Plot 5 (144.14 µg/L) and Plot 4 (140.12 µg/L) which had the least TPH concentration (Fig. 4.1). The n-alkanes accounted for more than fifty percent of the total TPH concentration in the entire plots, ranging from 17.55 – 285.72 µg/L (Table 4.24). The mean concentration of TPH in the control site was 5.54 µg/L.

The TPH results of TPH in water samples in the various plots varied for the hydrocarbon peak numbers. Plot 1 recorded highest concentration in C40 (242.7939 ppm) and lowest in C32 (0.1441 ppm) (Table 4.30). Total petroleum hydrocarbon was highest in C8 (55.3412 ppm) and lowest in C28 (0.10799 ppm) in Plot 2 (Table 4.31). Plot 3 recorded highest concentration in C9 (174.78438 ppm) and lowest concentration in C31 (0.1747 ppm) (Table 4.32). Plot 4 had highest concentration of 42.72444 ppm (C40) and lowest concentration of 0.02011 ppm (C25) (Table 4.33). Plots 5 and 6 recorded highest concentrations of TPH in C8 (47.75551 ppm) and C9 (176.32076 ppm) respectively while the lowest concentrations were in C31 (0.10091 ppm) and C35 (0.05755 ppm) respectively (Tables 4.34 and 4.35).

Table 4.23 Total Petroleum Hydrocarbons (TPH) Present in Soil in Plot 1 (mg/kg)

Hydrocarbon Peak No	R.T. (min)	Results (ppm)	Peakarea (counts)
C8	1.439	279.0567	504832
C9	1.961	86.2805	189330
C10	2.635	45.9457	99784
C11	3.363	44.2582	90260
C12	4.059	37.6839	81548
C13	4.751	23.7768	51553
C14	5.141	46.7962	99779
C15	5.417	18.7673	39651
C16	6.068	6.1024	13684
C17	6.670	11.7732	24692
Pr	6.676	19.8539	41470
C18	7.174	9.1259	18930
Ph	7.267	4.2966	8193
C19	7.765	22.4856	46780
C20	8.283	6.2327	12820
C21	8.710	36.4324	69846
C22	9.233	22.2866	42041
C23	9.707	17.2001	31111
C24	10.107	10.3193	17775
C25	10.524	5.4800	9232
C26	10.976	8.3595	13712
C27	11.359	4.1310	6432
C28	11.720	6.7498	10667
C29	12.139	12.4606	19682
C30	12.545	13.8202	23994
C31	12.805	3.4734	6221
C32	12.903	11.0461	18560
C33	13.226	7.6795	12924
C34	13.562	2.4444	3955
C35	13.971	19.5793	28828
C36	14.427	5.4490	4682
C37	15.007	2.6728	3442
C38	15.665	4.1323	4360
C39	16.538	21.3199	18683
C40	17.539	529.7107	6591
TOTAL		1407.1824	1676044

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.24: Total Petroleum Hydrocarbons Present in Soil Sample in Plot 2 (mg/kg)

Peak No	R.T (min)	Result (ppm)	Peak Area(counts)
C8	1.441	448.7936	811898
C9	1.811	1840.7954	4039365
C10	2.629	133.3282	289560
C11	3.352	7.9669	16248
C12	4.049	56.8181	122955
C13	4.765	43.0758	93397
C14	5.155	43.8183	93430
C15	5.441	23.5327	49719
C16	5.973	50.3427	112889
C17	6.595	42.0008	88088
Pr	6.809	14.1485	29553
C18	7.106	32.0077	66394
Ph	7.256	2.4890	4746
C19	7.763	24.2051	50358
C20	8.279	1.9078	3924
C21	8.705	29.6818	56904
C22	9.234	7.9683	15031
C23	9.700	16.2180	29335
C24	10.113	2.3437	4037
C25	10.535	2.5976	4376
C26	10.951	5.5037	9028
C27	11.357	2.9676	4621
C28	11.712	6.8685	10854
C29	12.112	6.4701	10220
C30	12.530	1.0554	1832
C31	12.819	3.5359	6333
C32	12.885	2.7026	4541
C33	13.217	6.9583	11711
C34	13.571	6.5527	10603
C35	13.976	2.6049	3835
C36	14.446	4.3988	3780
C37	15.012	6.4423	8296
C38	15.665	10.2831	10849
C39	16.455	18.5062	16218
C40	17.548	244.3564	3040
Total		3153.2466	6097968

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.25: Total Petroleum Hydrocarbons Present in Soil Sample in Plot 3 (mg/kg)

Peak No	R.T (min)	Result (ppm)	Peak Area (counts)
C8	1.440	633.9189	114802
C9	1.793	1563.2410	3430311
C10	2.248	1617.5858	3513048
C11	3.334	222.0102	452766
C12	4.050	63.0273	136391
C13	4.766	51.5608	111794
C14	5.133	104.5363	222893
C15	5.460	19.5390	41281
C16	6.091	3.9530	8864
C17	6.576	52.3048	109698
Pr	6.744	35.6930	74555
C18	7.199	4.8225	10003
Ph	7.263	2.9317	5590
C19	7.746	27.3550	56911
C20	8.250	1.2480	2567
C21	8.686	23.5818	45210
C22	9.264	2.2162	4181
C23	9.675	10.6981	19350
C24	10.116	0.6170	1063
C25	10.595	10.0505	16931
C26	10.966	0.8539	1401
C27	11.353	1.6597	2584
C28	11.747	3.0255	4781
C29	12.119	1.3594	2147
C30	12.543	3.3594	5833
C31	12.813	2.7600	4943
C32	12.892	1.6706	2807
C33	13.230	8.7925	14798
C34	13.547	7.9166	12810
C35	13.994	2.2306	3284
C36	14.432	3.5728	3070
C37	15.061	2.7259	3510
C38	15.615	0.9914	1046
C39	16.391	254.2298	222790
C40	17.549	62.5551	778
		4808.5938	9696791

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.26: Total Petroleum Hydrocarbons Present in Soil Sample in Plot 4

(mg/kg)

Peak No	R.T (min)	Result (ppm)	Peak Area (counts)
C8	1.441	491.8317	889757
C9	1.994	232.0103	509114
C10	2.618	1187.8043	2579655
C11	3.391	103.7240	211534
C12	4.082	53.2261	115182
C13	4.773	30.1596	65392
C14	5.145	61.7433	131650
C15	5.413	33.9242	71674
C16	6.074	25.4872	57153
C17	6.647	21.3151	44704
Pr	6.818	27.1426	56695
C18	7.206	14.0107	29063
Ph	7.248	7.0180	13383
C19	7.751	19.7983	41190
C20	8.234	11.0342	22696
C21	8.67	41.2809	79141
C22	9.228	14.0980	26594
C23	9.687	19.3691	35034
C24	10.11	10.3453	17820
C25	10.517	10.9045	18370
C26	10.938	10.5379	17285
C27	11.330	11.1347	17337
C28	11.738	4.8143	7608
C29	12.115	3.1996	5054
C30	12.536	10.0518	17452
C31	12.811	4.5957	8231
C32	12.887	4.6903	7881
C33	13.240	16.2074	27277
C34	13.560	4.7814	7737
C35	13.998	8.2959	12214
C36	14.436	10.2056	8770
C37	15.014	2.8493	3669
C38	15.671	2.3435	2472
C39	16.478	6.2460	5472
C40	17.550	7.1800	960
Totals		2593.3616	5165222

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.27: Total Petroleum Hydrocarbons Present in Soil Sample in Plot 5 (mg/kg)

Peak No	R.T (min)	Result (ppm)	Peak Area (counts)
C8	1.442	575.9320	1041900
C9	1.924	902.9778	1981457
C10	2.657	85.6985	186119
C11	3.364	37.2346	75936
C12	4.068	18.2648	39525
C13	4.757	4.2298	9171
C14	5.142	43.0403	91771
C15	5.414	12.7788	26998
C16	6.086	5.8632	13148
C17	6.643	14.9669	31390
Pr	6.768	48.8878	102115
C18	7.201	26.1495	54242
Ph	7.260	14.7324	28093
C19	7.756	29.2959	60949
C20	8.203	130.7825	26009
C21	8.726	167.5387	321195
C22	9.227	119.8192	226026
C23	9.706	93.3507	168850
C24	10.094	20.1164	34651
C25	10.553	7.5694	12751
C26	10.962	12.1327	19901
C27	11.339	31.0976	48420
C28	11.729	1.6414	2594
C29	12.126	11.9771	18919
C30	12.540	9.9102	17206
C31	12.788	9.3081	16670
C32	12.884	6.9665	11706
C33	13.217	10.5354	17731
C34	13.570	5.1400	8317
C35	13.958	15.9228	23444
C36	14.425	11.0152	9465
C37	15.007	4.4824	5772
C38	15.673	2.6427	2788
C39	16.504	10.1327	8880
C40	17.543	377.1524	4693
Total		2879.2861	4991802

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.28: Total Petroleum Hydrocarbons Present in Soil Sample in Plot 6 (mg/kg)

Peak No	R.T (min)	Result (PPM)	Peak Area (counts)
C8	1.444	708.7004	1282087
C9	1.839	1985.1532	4356138
C10	2.643	133.6002	290151
C11	3.368	16.4264	33500
C12	4.075	48.0636	104010
C13	4.796	6.9081	14978
C14	5.144	96.1897	205097
C15	5.444	24.4794	51719
C16	5.968	85.5794	191904
C17	6.586	59.1138	123979
Pr	6.798	15.1699	31686
C18	7.207	9.4428	19587
Ph	7.245	12.0195	22920
C19	7.753	51.2157	106552
C20	8.300	6.2061	12765
C21	8.693	61.5976	118091
C22	9.220	31.0600	58591
C23	9.693	45.4305	82173
C24	10.163	36.7802	63356
C25	10.605	23.2835	39224
C26	10.961	15.5384	25487
C27	11.383	12.4544	19392
C28	11.737	9.5346	15067
C29	12.092	13.6727	21597
C30	12.537	11.0250	19141
C31	12.797	6.1287	10976
C32	12.874	10.7068	17990
C33	13.221	18.8805	31775
C34	13.549	13.9372	22551
C35	13.976	4.5905	6759
C36	14.426	17.1112	14703
C37	15.028	13.0175	16764
C38	15.670	13.5289	14273
C39	16.512	19.7477	17306
C40	17.544	501.6814	6242
Total		4137.9761	7468531

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.29 Total Petroleum Hydrocarbons (TPH) Present in Control Soil (Ctr)

(mg/kg)

Peak No	R.T. (min)	Results (ppm)	Peak Area (counts)
C8	1.381	6.10230	60411
C9	1.850	2.01140	17930
C10	2.601	0.6234	37784
C11	3.342	1.50332.	80260
C12	4.008	1.00234.	41541
C13	4.701	0.11210	11452
C14	5.132	0.1321	68798
C15	5.392	1.2103	27612
C16	6.054	0.2021	23874
C17	6.650	1.2332	18762
Pr	6.681	1.0534	51270
C18	7.139	1.12110	32930
Ph	7.240	0.0021	1593
C19	7.699	0.1150	25710
C20	8.267	0.1317	22020
C21	8.705	1.5431	38844
C22	9.200	1.0016	42041
C23	9.701	1.20010	31111
C24	10.100	0.01931	17775
C25	10.520	0.01800	1232
C26	10.925	0.00005	1712
C27	11.350	1.1310	1632
C28	11.696	0.0098	2367
C29	12.131	0.00006	12348
C30	12.521	0.3202	21238
C31	12.801	0.20011	3672
C32	12.882	1.04610	1156
C33	13.215	0.1115	12924
C34	13.553	0.12004	3955
C35	13.792	0.00003	1811
C36	14.389	0.20490	4682
C37	15.000	0.00112.	3442
C38	15.499	0.01131	21211
C39	16.398	1.13100	1117
C40	17.481	2.5101	11233
TOTAL		25.82121	757450

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.30: Total Petroleum Hydrocarbons Present in Water Sample in Plot 1(μg/L)

Peak No	R.T.(min)	Result (ppm)	Peak Area (counts)
C8	1.440	52.2579	945382
C9	2.082	47.8741	1050531
C10	2.648	12.6941	275689
C11	3.352	7.4403	151738
C12	4.096	5.9799	122914
C13	4.787	0.9526	20656
C14	5.144	9.6779	206354
C15	5.440	1.9781	41794
C16	6.099	0.8515	19096
C17	6.585	5.6838	19096
Pr	6.771	1.4177	29614
C18	7.190	0.3020	6265
Ph	7.257	0.3230	6160
C19	7.756	3.3774	70267
C20	8.264	.9.4023	19340
C21	8.694	3.5159	67405
C22	9.218	0.6677	12596
C23	9.681	1.8497	33457
C24	10.160	0.4926	8486
C25	10.575	.7.9582	13406
C26	10.976	0.4221	6925
C27	11.346	0.5628	8763
C28	11.739	0.4841	7652
C29	12.124	0.3373	5329
C30	12.547	0.1995	3465
C31	12.809	0.2584	4628
C32	12.902	0.1441	2422
C33	13.238	0.6596	11102
C34	13.569	0.4732	7657
C35	13.953	0.4812	7085
C36	14.440	1.5450	13277
C37	14.986	1.2454	16038
C38	15.668	0.7908	8343
C39	16.497	5.4487	4775
C40	17.545	242.7939	3021
Total		191.201	3330838

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.31: Total Petroleum Hydrocarbons Present in Water Sample in Plot 2 (μg/L)

Peak No	R.T (min)	Result (PPM)	Peak Area (counts)
C8	1.440	55.3412	1001161
C9	1.980	18.4484	404824
C10	2.648	3.11359	67621
C11	3.345	8.26214	168498
C12	4.094	3.44799	74615
C13	4.770	6.63928	143952
C14	5.138	12.0891	257766
C15	5.399	2.23970	47319
C16	6.025	3.18976	71527
C17	6.650	2.25564	47307
Pr	6.820	2.13896	44678
C18	7.166	0.93087	19309
Ph	7.236	1.01728	19399
C19	7.753	2.12408	44191
C20	8.263	1.69433	34851
C21	8.691	4.17334	80009
C22	9.217	0.30960	5840
C23	9.677	2.93334	53057
C24	10.114	0.21305	3670
C25	10.528	0.14970	2522
C26	10.961	0.17721	2907
C27	11.373	0.16734	2606
C28	11.729	0.10799	1707
C29	12.111	0.25207	3982
C30	12.529	0.44376	7704
C31	12.780	0.77736	13922
C32	12.917	0.89418	15025
C33	13.211	0.43752	7363
C34	13.537	0.41003	6635
C35	13.968	1.07546	15835
C36	14.430	1.23988	10654
C37	14.999	0.44551	5737
C38	15.675	0.80582	8501
C39	16.484	1.51438	13271
C40	17.553	48.8357	6077
Total		188.2956	2714042

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.32: Total Petroleum Hydrocarbons Present in Water Sample in Plot 3

   (µg/L)

Peak No	R.T. (min)	Result (ppm)	Peak Area (counts)
C8	1.440	59.67527	1079566
C9	1.797	174.78438	3835396
C10	2.643	10.27400	223129
C11	3.406	12.48095	254536
C12	4.077	21.7093	46979
C13	4.818	4.08042	88471
C14	5.138	11.94574	254708
C15	5.375	7.68914	162453
C16	6.098	1.66895	37425
C17	6.581	7.57401	158849
Pr	6.814	1.17745	24594
C18	7.199	0.29060	6028
Ph	7.249	1.18630	22622
C19	7.751	5.17090	107579
C20	8.262	0.43898	9030
C21	8.690	4.11333	78858
C22	9.214	0.30309	5717
C23	9.678	2.56279	46355
C24	10.115	0.39590	6820
C25	10.533	0.16736	2819
C26	10.948	0.71574	11740
C27	11.375	0.49326	7680
C28	11.711	0.40817	6450
C29	12.106	0.56593	8639
C30	12.534	0.18004	3126
C31	12.804	0.17472	3129
C32	12.902	0.44965	7555
C33	13.218	0.81106	13650
C34	13.565	0.79553	12872
C35	13.972	0.30520	4494
C36	14.138	0.66234	5691
C37	14.992	0.18193	2343
C38	15.668	1.50714	15900
C39	16.526	1.52351	13351
C40	17.558	35.08817	4366
Total		352.01287	6573220

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.33: Total Petroleum Hydrocarbons Present in Water Sample in Plot 4

(µg/L)

Peak No	R.T. (min)	Result (ppm)	Peak Area (counts)
C8	1.879	9.51500	172133
C9	2.366	3.00627	65968
C10	2.642	1.98571	43125
C11	3.364	0.47620	9712
C12	4.071	0.54386	11769
C13	4.757	0.51090	11077
C14	5.140	1.51003	32197
C15	5.424	0.42951	9075
C16	6.039	0.32950	7389
C17	6.656	25967	5446
Pr	6.787	0.35369	7388
C18	7.190	0.16627	3449
Ph	7.263	0.27886	5318
C19	7.730	0.96888	20157
C20	8.251	0.33688	6929
C21	8.754	0.12092	2318
C22	9.224	0.06656	1256
C23	9.706	0.06656	1409
C24	10.121	0.07787	346
C25	10.556	0.02011	2171
C26	10.957	0.12885	553
C27	11.367	0.03372	381
C28	11.724	0.02450	584
C29	12.112	0.03697	549
C30	12.542	0.03473	4697
C31	12.813	0.27054	3416
C32	12.888	0.19075	4341
C33	13.219	0.25833	6290
C34	13.550	0.37373	7286
C35	13.982	0.45031	11402
C36	14.427	0.77444	7805
C37	15.011	0.90830	6748
C38	15.677	0.52403	7786
C39	16.481	0.73798	3978
C40	17.548	42.72444	5316
Total		68.88228	489764

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.34: Total Petroleum Hydrocarbons Present in Water Sample in Plot 5 (μg/L)

Peak No	R.T. (min)	Result (ppm)	Peak Area (counts)
C8	1.441	47.75551	863929
C9	2.013	15.6996	344506
C10	2.664	11.39096	247387
C11	3.418	11.2454	229340
C12	4.068	1.30213	28178
C13	4.723	4.13500	89655
C14	5.146	4.89557	104384
C15	5.448	2.94554	62232
C16	6.003	3.28536	73671
C17	6.582	3.22047	67543
Pr	6.822	1.10926	23170
C18	70184	1.03848	21541
Ph	7.223	2.12722	40564
C19	7.762	2.36609	49226
C20	8.221	1.34050	27573
C21	8.700	3.89673	74706
C22	9.229	0.47716	9001
C23	9.686	1.33198	24092
C24	10.155	0.71358	12292
C25	10.597	1.35968	22905
C26	10.962	5.2824	8665
C27	11.371	0.13090	2038
C28	11.746	0.636990	10112
C29	12.125	0.42310	6683
C30	12.544	0.18327	3182
C31	12.815	0.10091	1807
C32	12.880	0.57248	9619
C33	13.222	0.71404	12017
C34	13.573	0.86276	13960
C35	13.983	0.14090	2074
C36	14.416	1.98766	17080
C37	14.986	0.38596	4970
C38	15.684	0.39144	4130
C39	16.481	0.79228	6943
C40	17.551	14.65627	1824
Total		144.11644	2520999

R.T- Retention time, ppm- part per million, Pr- pristine, Ph-phytane

Table 4.35: Total Petroleum Hydrocarbons Present in Water Sample in Plot 6

(μg/L)

Peak No	R.T. (min)	Result (ppm)	Peak Area (counts)
C8	1.440	62.48516	1130399
C9	1.790	176.32076	3869110
C10	2.647	4.86991	105764
C11	3.361	17.00835	346867
C12	4.055	2.82123	61052
C13	4.682	9.79779	212434
C14	5.132	12.42933	265020
C15	5.388	4.82948	102035
C16	6.078	0.37190	8339
C17	6.576	7.01176	147057
Pr	6.815	0.66611	13914
C18	7.195	0.26523	5505
Ph	7.250	1.23177	23489
C19	7.745	4.19735	87324
C20	8.247	0.23667	4868
C21	8.735	1.46602	28106
C22	9.213	0.54526	10286
C23	9.672	1.82448	33001
C24	10.089	0.54118	9322
C25	10.527	0.83589	14081
C26	10.975	0.13782	2261
C27	11.372	0.38576	6006
C28	11.727	0.10962	1732
C29	12.108	0.18555	2931
C30	12.540	0.17032	2957
C31	12.799	0.05755	1031
C32	12.896	0.36255	6092
C33	13.195	0.36670	6171
C34	13.554	0.04553	737
C35	13.935	1.08656	15998
C36	14.432	0.95476	8204
C37	15.028	0.90300	11629
C38	15.672	0.60514	7017
C39	16.560	0.56005	4908
C40	17.543	9.47887	1179
Total		325.2254	6556823

R.T- Retention time, ppm- part per million, Pr- pristane, Ph-phytane

Table 4.36 Total Petroleum Hydrocarbons (TPH) Present in Water Source (control) (µg/L)

Peak No	R.T. (min)	Results (ppm)	Peak area (counts)
C8	1.402	1.90110	70211
C9	1.765	0.51140	27830
C10	2.710	0.1200	15774
C11	3.297	0.10312.	40290
C12	4.028	0.00234.	61571
C13	4.690	0.11210	21432
C14	5.147	0.1321	58698
C15	5.430	0.02103	47112
C16	6.152	0.0011	13270
C17	6.600	0.0332	14701
Pr	6.681	0.0124	41232
C18	7.220	0.0110	22910
Ph	7.227	0.0011	2520
C19	7.679	0.0140	18720
C20	8.237	0.0217	11090
C21	8.705	0.3411	22104
C22	9.215	0.0018	32031
C23	9.761	0.0001	2326
C24	10.180	0.01200	20021
C25	10.531	0.01100	2102
C26	10.889	0.00001	1340
C27	11.353	0.0011	1323
C28	11.698	0.0018	2128
C29	12.121	0.00010	21158
C30	12.531	0.0201	16660
C31	12.811	0.10010	2765
C32	12.870	0.00110	1050
C33	13.220	0.0121	11119
C34	13.555	0.00054	2760
C35	13.783	0.00013	2810
C36	14.378	0.02110	3451
C37	15.109	0.01320	3120
C38	15.501	0.01120	20570
C39	16.399	0.5010	1005
C40	17.471	1.50032	11237
TOTAL		5.54417	648440

R.T- Retention time, ppm- part per million, Pr- pristine, Ph-phytane

4.1.25 Summary of concentrations of various groups of TPH present in soil and water samples

The concentration of TPH in soil and water were grouped based on their carbon length, persistence and toxicity. The results obtained showed that there were more concentrations of short chain hydrocarbon than the long chain hydrocarbon in both soil and water samples. The n-alkanes contributed more to the TPH concentrations in the plots. The C23-C40 group (long chain hydrocarbons) had the second highest concentration of TPH in the plots. Long chain hydrocarbons which are known for their persistence and toxicity in the environment had elevated concentration in the plots. The concentrations of TPH in soil and water were in the order; $\sum$ (n-alkanes) >$\sum$ (C23-C40) (C15-C19) >$\sum$ (C18-C22) in both soil and water samples. The order of concentrations of TPH in the various plots were; Plot 3 >Plot 6 > Plot 2 > Plot 5 > Plot 4 >Plot 1 and Plot 6 > Plot 3 > Plot 1 > Plot 2 >Plot 5 > Plot 4 in soil and water respectively (Tables 4.37 and 4.38).

Table4.37: Summary of Concentrations of Various Groups of TPH Present in Soil Samples in the Plots

Parameters	Plot1	Plot2	Plot3	Plot4	Plot5	Plot6	Ctr
$\sum$(n-alkanes)	1501.35	2511.20	4285.85	2160.47	1667.34	2995.01	4.16
$\sum$ (C15-C19)	74.65	188.71	146.85	178.66	152.63	256.97	5.02
$\sum$ (C18-C22)	91.24	98.22	62.13	107.20	488.28	171.51	2.11
$\sum$ (C23-C40)	355.52	356.11	314.04	147.30	571.03	714.48	14.53
Total	1407.18	3153.25	4808.59	2593.36	2879.27	4137.98	25.82

Key: Ctr = control

Table 4.38: Summary of Concentrations of Various Groups of TPH Present in Water Samples in the Plots

Parameters	Plot1	Plot2	Plot3	Plot4	Plot5	Plot6	Ctr
$\sum$(n-alkanes)	136.75	107.33	275.40	17.55	96.42	285.72	0.08
$\sum$ (C15-C19)	13.93	13.89	24.75	2.78	16.09	18.57	1.05
$\sum$ (C18-C22)	10.97	10.25	11.50	1.93	11.24	7.94	1.48
$\sum$ (C23-C40)	29.73	56.82	63.14	46.62	20.39	12.99	2.93
Total	191.20	188.29	325.01	68.88	144.14	325.22	5.54

Key: Ctr = control

4.1.26 Concentrations of USEPA priority PAHs present in soil samples

The results of the concentrations of the priority PAHs (mg/kg) investigated in the various plots are depicted in Table 4.39. Among the low molecular weight (LMW) PAHs, Phenanthrene (Phe) had the highest concentration and was detected in all plots except in the control site. The concentration of Phe ranged from 324 mgkg^{-1} to 584 mgkg^{-1} in the various plots with the highest concentration recorded in Plot 2. Fluorene was not detected in all the plots except in Plots which had a concentration of 116.00 mgkg^{-1}. 1-Methylnaphthalene recorded a total concentration of 16 mgkg^{-1} in Plot 3 only which was the lowest total concentration of LMW PAH in the plots (Table 4.39).

Among the 16 priority PAHs investigated, 4 high molecular weight (HMW) PAH compounds (Fluoranthene, Benzo(k) fluoranthene, Benzo(a)pyrene and Benz(a)anthracene) were not detected (ND). Pyrene and Chrysene were only detected in Plot 2 with concentrations of 12.00 mgkg^{-1} and 4.11 mgkg^{-1} respectively. Benzo(b) fluoranthene had the highest concentration of HMW PAHs which ranged from 4.00 mgkg^{-1} to 244.00 mgkg^{-1} in the various plots.

Three carcinogenic PAHs were reported in this study. Among the carcinogenic PAHs, Dibenz(a, h) anthracene had the highest PAH concentration which ranged from 32.00 mgkg^{-1} to 184 mgkg^{-1} in the entire plots. Other carcinogenic PAHs detected in low concentrations were Chrysene (4.11 mgkg^{-1}) and Indeno(1,2,3-cd) pyrene (4.00 mgkg^{-1} to32.00 mgkg^{-1})**.**

The numbers of PAH compounds reported in variuos plots differed. Plots 1, 2 and 3 had 8 PAH compounds each, Plots 4 and 5 had 4 PAH compounds each while Plot 6 had 6 PAH compounds. The control sample recorded no PAHs. Statistical analysis showed

that the concentrations of LMW PAHs were more than that of HMW PAHs with C-PAH having the least concentration of PAHs in the plots.

4.1.27 Concentrations of USEPA priority PAHs present in water samples

The results of the concentrations of the priority PAHs (µg/mL) investigated in water samples from the various plots are depicted in Table 4.40. The range of concentrations of LMW PAHs in the plots were, Naphthalene 0.0929 – 0.2092 µg/mL, Acenaphthylene ND – 0.0061 µg/mL, Acenaphthene 0.0001 – 0.0008 µg/mL, Fluorene ND – 0.0029 µg/mL, Phenanthrene ND – 0.0016 µg/mL and Anthracene ND – 0.0211 µg/mL. The highest concentration of LMW PAH was recorded in 1-Methylnaphthalene (Table 4.40).

The range of high molecular weight PAHs (µg/mL) detected include, Benz(a) anthracene ND – 0.0067, Chrysene ND – 0.0001, Benzo(b)fluoranthene ND – 0.0033, Benzo(k)fluoranthene ND – 0.0011, Indeno(1,2,3-cd)pyrene ND – 0.0008 and Dibenz(a,h) anthracene ND – 0.0028. Fluorene, Pyrene, Benzo (a) pyrene and Benzo(g,h,i) perylene were not detected in any of the plots.

The mean of the total concentrations of 5 carcinogenic PAH compounds detected include; Chrysene 0.0003, Benz(a) anthracene 0.0012, Benzo(k)fluoranthene 0.0003, Indeno(1,2,3-cd)pyrene 0.0002 and Dibenz(a,h) anthracene0.0014. There were more concentrations of LMW PAHs than the HMW PAHs in the samples. Plots 2,3 and 6 had the highest numbers (10) of detectable PAHs compounds while Plot 4 had the least number (7)(Table 4.40).

Table 4.39: Concentration of Priority PAHs in Soil Samples in the Various Plots

PAHs (mg/kg)	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Ctr
1-Methylnaphthalene	ND	ND	16.00	ND	ND	ND	ND
2-Methylnaphthalene	20.03	8.00	8.00	36.00	8.00	8.05	ND
Acenaphthylene	ND	84.00	84.09	152.00	64.00	172.00	ND
Acenaphthene	4.00	ND	ND	4.00	4.00	28.00	ND
Fluorene	ND	ND	ND	ND	116.00	ND	ND
Phenanthrene	428.00	584.03	324.00	452.00	376.00	388.00	ND
Anthracene	16.00	112.00	ND	32.00	ND	ND	ND
Fluoranthene	ND	ND	ND	ND	ND	ND	ND
Pyrene	ND	12.00	ND	ND	ND	ND	ND
Benz(a)anthracene	ND	ND	ND	ND	ND	ND	ND
Chrysene	ND	4.11	ND	ND	ND	ND	ND
Benzo(b)fluoranthene	52.00	244.00	40.00	32.00	4.00	172.00	ND
Benzo(a)pyrene	ND	ND	ND	ND	ND	ND	ND
Benzo(k)fluoranthene	ND	ND	ND	ND	ND	ND	ND
Indeno(1,2,3-cd)pyrene	4.00	32.00	28.00	ND	4.00	ND	ND
Dibenz(a,h)anthracene	144.00	ND	44.00	32.00	ND	184.10	ND
Benzo(g,h,i)perylene	20.00	ND	8.00	ND	ND	ND	ND

ND – not detected, mg/kg – milligram per kilogram, Ctr - control

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Table 4.40: Concentrations of USEPA Priority PAHs Present in Water Samples in

the Various Plots

PAHs (µg/mL)	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Ctr
1-Methylnaphthalene	0.1371	0.2092	0.1576	0.1809	0.1024	0.1298	ND
2-Methylnaphthalene	0.1242	0.3312	0.1426	0.1636	0.0929	0.1176	ND
Acenaphthylene	0.004	0.0031	ND	0.0028	0.0061	0.0028	ND
Acenaphthene	0.0004	0.0008	0.0001	0.0001	0.0002	0.0002	ND
Fluorene	0.0024	0.0048	0.0017	0.0036	ND	0.0029	ND
Phenanthrene	0.0005	0.0016	0.004	ND	0.0012	0.0006	ND
Anthracene	0.0003	0.0211	0.0024	ND	0.0007	0.0004	ND
Fluoranthene	ND	ND	ND	ND	ND	ND	ND
Pyrene	ND	ND	ND	ND	ND	ND	ND
Benz(a)anthracene	0.002	ND	0.0067	ND	0.001	ND	ND
Chrysene	ND	ND	0.0001	ND	0.0001	ND	ND
Benzo(b)fluoranthene	ND	0.0033	0.0022	ND	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND	0	ND	ND
Benzo(k)fluoranthene	ND	0.0008	ND	0.0011	ND	0.0001	ND
Indeno(1,2,3-cd)pyrene	ND	ND	0.0004	ND	ND	0.0008	ND
Dibenz(a,h)anthracene	0.0008	0.003	ND	0.0019	ND	0.0028	ND
Benzo(g,h,i)perylene	ND	ND	ND	ND	ND	ND	ND

µg/mL- microgram per kilogram, ND- not detected, Ctr- control

4.1.28 Degradation of priority PAH by *Aspergillus niger* strain ATCC 1015

The GC-MS showed that *Aspergillus niger* mineralised the 16 priority PAHs at different rates as shown in Table 4.41. It was observed that *A. niger* strain ATCC 1015 completely metabolised 4 of the 16 priority PAHs. These include, Naphthalene, Dibenz(a,h,)Anthracene, Indeno(1,2,3,-cd)pyrene, and Benzo(g,h,I,)perylene. Among the LMW PAHs, the least utilised was Fluorene (68 %) while the least utilised in the HMW PAHs was Chrysene (49.5 %). Statistical analysis showed that *A. niger* degraded 72.11 % of the PAHs after 21 days.

4.1.29 Degradation of 16 priority PAH by screened isolate of *Penicillium notatum* strain P72-30

The results of the degradation of priority PAHs by *Penicillium notatum* strain P72-30 as revealed by GC-MS analysis are presented in Table 4.42. There were more utilisation of LMW PAHs than HMW PAHs by the organism. There were complete mineralistion of FLA, DBA and InP in the medium. Among the LMW PAHs, the least utilised was NAP (39.00 %) while the least utilised in the HMW PAHs was BaP (22.00 %). *P. notatum* utilised to varying degrees all the PAHs in the medium and had a total degradation rate of 63.65 % (Table 4.42).

4.1.30 Degradation of 16 priority PAH by *Pseudomonas aeruginosa* strain PA14

*Pseudomonasaeruginosa*strainPA14showed high utilisation capacity on the 16 priority PAHs pollutants. Four of the pollutants (FLA, BgP, InP, DBA) were mineralised by the isolate. *P. aeruginosa* showed relatively strong affinity to both LMW and HMW PAHs in the medium with least utilisation on NAP (70.50 %) and CHR (71.00 %) for LMW PAHs and HMW PAHs respectively. The total percentage utilisation for *Pseudomonas aeruginosa* strain PA14 was 84.80 %.

4.1.31 Degradation of 16 priority PAH by *Bacillus thuringiensis* strain M43

The results of the degradation of priority PAHs by *Bacillus thuringiensis* strain M43 as revealed by GC-MS analysis are presented in Table 4.44. There were more utilisation of LMW PAHs than HMW PAHs by *B. thuringiensis*. There were complete mineralistion of NAP, DBA BgP and InP in the medium. The least utilised PAHs in the medium by *B. thuringiensis* were BbF (0.50 %), CHR (2.50 %) and BkF (43.00 %). *Bacillus thuringiensis* recorded a total percentage utilisation of 65.75 % on the PAHs priority pollutants.

Table 4.41: Biodegradation of Prioty PAH by *Aspergillus niger* strain ATCC 1015

PAH compounds	R.T	QIon	Response	Conc.	Dev(Min) Qvalue	Percentage Degraded
Benzene,1,2,3trimethyl	6.002	105	18701	1.05	17	47.50
Naphthalene	0.000		0	N.D		100
2-Methylnaphthalene	8.511	128	5054	0.58	1	71.00
Acenaphthylene	8.511	128	5054	0.58	1	71.00
Acenaphthene	9.698	142	850	0.43	43	78.50
Fluorene	10.129	142	6830	0.64	60	68.00
Anthracene	11.694	152	6488	0.63	1	68.50
Phenanthrene	12.282	153	5598	0.59	58	70.50
Fluoranthene	13.411	166	8337	0.69	64	65.5
Pyrene	15.652	178	7299	0.65	1	67.50
Benz(a)anthracene	16.094	178	8186	0.68	1	66.00
Chrysene	19.982	202	17631	1.01	1	49.50
Benzo(b) Fluoranthene	20.768	202	10924	0.78	1	61.00
Benzo(k) Fluoranthene	24.312	228	13342	0.86	1	57.00
Benzo(a) Pyrene	24.568	2228	13586	0.87	11	56.50
Dibenz(a,h) Anthracene	0.000		0	N.D		100
Indeno (1,2,3-cd) pyrene	0.000		0	N.D		100
Benzo (g, h, i) perylene	0.000		0	N.D		100
Total				10.04		72.11

R.T- Retention time, QIon- qaulifier ion, conc.- concentration, Dev - deviation

Table 4.42: Degradation of Priority PAH by *Penicillium notatum* strain P72-30

Target compounds	R.T	QIon	Response	Conc	Dev(Min) Qvalue	Percentage Degraded
Benzene,1,2,3-trimethyl	5.810	105	47204	1.64	53	18.00
Naphthalene	6.241	105	23618	1.22	28	39.00
2-Methylnaphthalene	8.394	128	9282	0.72	1	64.00
Acenaphthylene	8.761	128	16407	0.97	1	51.50
Acenaphthene	9.977	142	4247	0.55	1	72.50
Fluorene	10.088	142	9398	0.73	1	63.50
Anthracene	11.945	152	9126	0.72	1	64.00
Phenanthrene	12.212	153	6923	0.64	54	68.00
Fluoranthene	0.000		0	N.D		100
Pyrene	15.867	178	13593	0.87	1	56.50
Benz(a)anthracene	15.978	178	4600	0.56	1	72.00
Chrysene	20.046	202	38513	1.74	3	13.00
Benzo(b) Fluoranthene	20.686	202	19657	1.08	1	46.00
Benzo(k) Fluoranthene	24.202	228	20696	1.12	1	44.00
Benzo(a) Pyrene	24.144	228	33426	1.56	33	22.00
Dibenz(a,h) Anthracene	0.000		0	N.D		100.00
Indeno (1,2,3-cd) pyrene	0.000		0	N.D		100.00
Benzo (g, h, i) perylene	0.000		0	0.01		99.50
Total				13.05		63.65

R.T- Retention time, QIon- qaulifier ion, conc.- concentration, Dev - deviation

Table 4.43: Degradation of Priority PAHs by *Pseudomonas aeruginosa* strain PA14

PAH compounds	R.T	QIon	Response	Conc	Dev(Min) Qvalue	Percentage Degraded
Benzene,1,2,3trimethyl	0.000		0	N.D.		100.00
Naphthalene	6.299	105	55432	0.59	48	70.50
2-Methylnaphthalene	8.272	128	5720	0.42	1	79.00
Acenaphthylene	8.517	128	12346	0.44	12	78.00
Acenaphthene	9.919	142	6416	0.42	52	79.00
Fluorene	9.919	142	7750	0.43	1	78.50
Anthracene	11.881	152	13282	0.45	1	77.50
Phenanthrene	12.439	153	5066	0.42	43	79.00
Fluoranthene	13.405	166	4132	0.41	78	79.50
Pyrene	15.617	178	9833	0.43	1	78.50
Benz(a)anthracene	0.000		0	N.D.		100.00
Chrysene	19.941	202	52845	0.58	1	71.00
Benzo(b) Fluoranthene	20.535	202	10489	0.44	1	78.00
Benzo(k) Fluoranthene	0.000		0	N.D.		100.00
Benzo(a) Pyrene	24.580	228	11551	0.44	1	78.00
Dibenz(a,h) Anthracene	0.000		0	N.D.		100.00
Indeno (1,2,3-cd) pyrene	0.000		0	N.D.		100.00
Benzo (g, h, i) perylene	0.000		0	N.D.		100.00
Total				5.47		84.80

R.T- Retention time, QIon- qaulifier ion, conc.- concentration, Dev - deviation

Table4.44: Degradation of Priority PAH by *Bacillus thuringienis* strain M43

Target compounds	R.T	QIon	Response	Conc	Dev(Min) Qvalue	Percentage Degraded
Benzene,1,2,3trimethyl	0.000		0	N.D.		100.00
Naphthalene	0.000		0	N.D.		100.00
2-Methylnaphthalene	8.278	128	3254	0.51	1	94.5
Acenaphthylene	8.714	128	15480	0.94	1	73.00
Acenaphthene	9.936	142	7391	0.66	26	87.00
Fluorene	9.936	142	10308	0.76	7	82.00
Anthracene	11.956	152	14142	0.89	59	75.50
Phenanthrene	12.474	153	4947	0.57	1	91.50
Fluoranthene	13.475	166	6147	0.61	18	89.50
Pyrene	15.483	178	9023	0.71	32	84.50
Benz(a)anthracene	15.832	178	9204	0.72	1	84.00
Chrysene	19.842	202	44735	1.95	21	2.50
Benzo(b) Fluoranthene	20.401	202	56151	1.99	15	0.50
Benzo(k) Fluoranthene	24.376	228	21349	1.14	1	43.00
Benzo(a) Pyrene	24.597	228	13895	0.88	54	56.00
Dibenz(a,h) Anthracene	0.000		0	N.D.		100.00
Indeno (1,2,3-cd) pyrene	0.000		0	N.D.		100.00
Benzo (g, h, i) perylene	0.000		0	N.D.		100.00
Total				12.33		65.75

R.T- Retention time, QIon- qaulifier ion, conc.- concentration, Dev - deviation

4. 1.32 Identification of bacterial and fungal genes for PAH degradation

Identification of bacterial and fungal genes were based on the ability of the PAH primers to amplify the gene sequence of the isolates when subjected to gel electrophoresis. Two sets of primers were used for the amplification and identification of the bacterial and fungal genes. Among the two bacterial isolates, each bacterium was amplified by only one of the two primers used. Gel electrophoresis of *Bacillus thuringiensis* strain M43 revealed that part the gene sequence of the bacterium (Appendix D)was amplified at 580 bp by pahE1 primer (Plate 4.3) while that of *Pseudomonas aeruginosa strain* PA14 (Appendix D) was amplified by pahE2 primers. Both fungal isolates' (*Aspergillus niger* strain ATCC 1015 and *Penicillium notatum* strain P72-30) gene sequences (Appendix D) were amplified by pahE1 primer only (Plates 4.4 and 4.5). All the isolates were amplified at 580 bp.
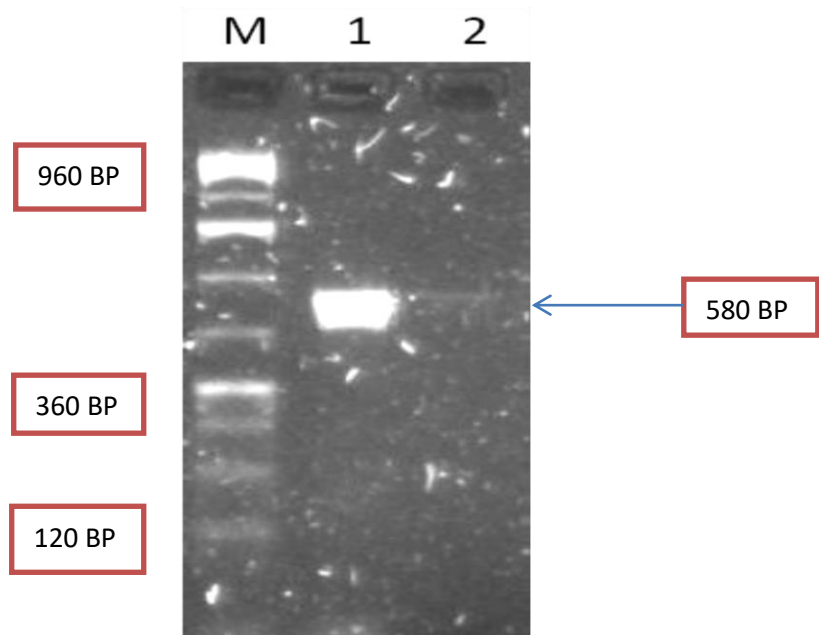
Plate III: **Gel Electrophoresis of Amplified DNA of** *Bacillus thuringiensis* **strain M43 with pahE1**
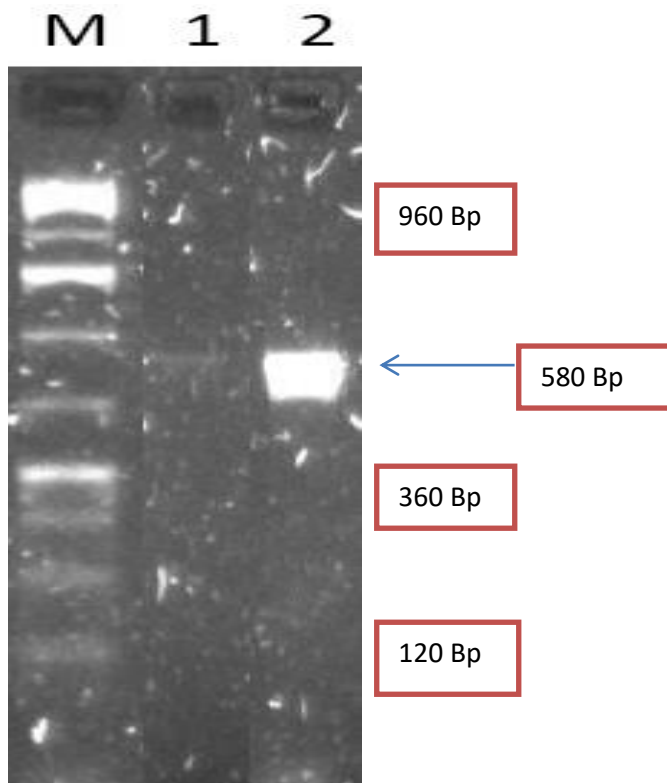
Plate IV: Gel Electrophoresis of Amplified DNA of *Pseudomonas aeruginosa strain PA14* with pahE2
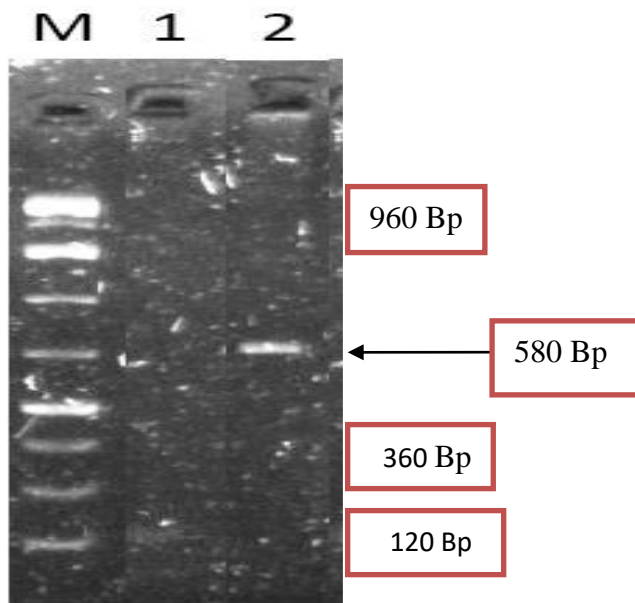
Plate V: Gel Electrophoresis of one Amplified DNA of *Penicillium notatum* strain P72-30 with pahE1

M 1 2

960Bp

580 Bp

360 Bp

120 Bp
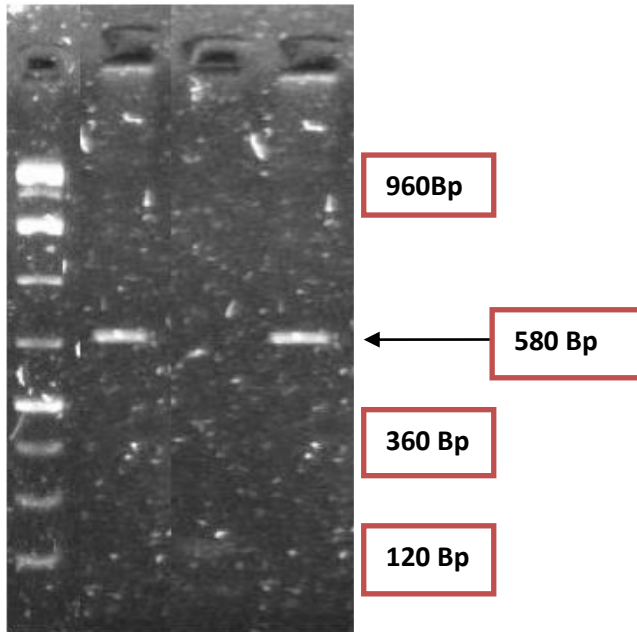
Plate VI: Gel Electrophoresis of Amplified DNA of *Aspergillus niger* **strain ATCC 1015 using pahE1**

Table 4.45: Detection of PAH-degrading Gene in Bacterial and Fungal Isolates

Isolate name	Strain	PAH degrading gene		Gene Bank accession No
		pahE1	pahE2	
Bacillus thuringiensis	M43	+	_	KY307913.1
Pseudomonas aeruginosa	PA14	_	+	KY963575.1
Penicillium notatum	P72-30	+	_	ATCC16834
Aspergillus niger	ATCC 1015	+	_	KJ439163.1

+ sign indicates the PCR product was detected, – sign indicates PCR product was not detected.

4.2   Discussion

The total mean counts of aerobic heterotrophic bacteria (AHB) in soil were greater than the counts obtained in water sample. This may be due to the fact that the soil is not mobile on its own, as such supports various activities of man as well as other living things, which eventually leads to the deposition of organic substances that help in providing nutrients for microbial proliferation. It could also be as a result of nutrient availability, which is usually more in soil when compared to water. Among the fungi isolated in this study, *Penicillium notatum* was prominent across all plots sampled. Elemuo *et al.* (2019) reported the versatility of *P. notatum* in the environment with ability to produce various enzymes, which help the microorganisms degrade crude oil in contaminated environment.

Plot 3 (Depot premises) had the highest mean counts of aerobic heterotrophic bacteria among the plots in the rainy season. This may be due to the availability of nutrients to the various groups of microorganisms enumerated and other favourable environmental conditions. The highest aerobic heterotrophic bacterial counts obtained during the rainy season is in concordance with the report of Olukunle (2013) and Ikuesan (2017) that moisture is one of the factors that influence microbial proliferation in soil. A dry soil can cause desiccation of microbial cells thereby reducing the overall microbial activities and causes some cells to be dormant. The soil can also hold substances in stationary mode allowing microorganisms to act on them conveniently without any disturbance unlike the liquid medium (Teknikio *et al*., 2019). The reduction in the AHB counts in water samples may be due to the fact that oxygen concentration in water is lower compared to soil sample, which may be due to presence of pore spaces that allows diffusion of atmospheric oxygen into them, thus enhancing bacterial proliferation in the soil (Ikuesan, 2017).

The low mean total coliform bacterial (TCB) counts generally observed in this study may be due to the inability of coliform bacteria to produce spores, which have been noted in the past to help microorganisms survive in environment that are not favourable for their existence and metabolisms. Moreover, there is paucity of information concerning the low proliferation of coliform bacteria on environment polluted by crude oil.

The mean total fungi (TF) counts in the soil and water sample in this study was highest in Plot 2, which is closer to the petroleum products depot both in wet and dry seasons. This may be due to the ability of fungi to tolerate adverse environmental conditions, especially with the formation of resistant spores.

The total mean counts of hydrocarbon utilising bacteria (HUB) and fungi (HUF) were lowest in the control soil both in wet and dry seasons. This shows that the microbial population found in communities surrounding petroleum products depot have the ability to utilise hydrocarbon due to periodic exposure to petroleum products. Though, there was no established pattern of growth observed in the microbial community in this study, their counts were higher than in the control. The total mean counts of HUB and HUF were generally low in the water sample in rainy and dry seasons compared to the soil sample. This is because, nutrient and oxygen availability in water is quite low compared to that of the soil sample. Different microbial genera use different pathways in the degradation of petroleum products, some of which take little time to complete a degradation process whereas others take longer time. Also, the types of enzymes utilised by microorganisms as well as the constituent of the petroleum products plays a great role in influencing biodegradation processes (Das *et al*., 2011).

Microbial population and diversity are usually high in a pristine environment unlike an environment experiencing influx of contaminants from crude oil spills. The reduction and changes in the microbial communities occur as a result of the inability of the indigenous microorganisms to withstand such extreme conditions and those that are able to withstand and even strive often have machineries and pathways they utilise to achieve that (Rampelotto, 2010, Das *et al.,* 2011, Jain *et al*., 2011). The indigenous bacterial diversity obtained in this study is similar to the one obtained by Nkiru *et al.* (2019).

This study reported some microorganisms that are peculiar to crude oil polluted environment, in which the genera *Bacillus* had the highest number of species, which include *B. subtilis, B. thurengiensis* and *B. licheniformis.* These bacteria in addition to *Pseudomonas aeruginosa* were all isolated from all the plots sampled. The dominant presence of *Bacillus* and *Pseudomonas* in this study supports the fact established by Osarumwense *et al.* (2019) that these bacterial genera efficiently degrade hydrocarbons in the environment through the production of surfactants and enzymes such as lipase. Agu *et al.* (2015) reported that spore forming ability of *Bacillus* helps the organisms in resisting toxic effects exerted by the petroleum compounds.

The results of the 16 priority PAH investigated in the soil samples in the plots showed contamination and pollution of the plots by PAH pollutnts to varying concentrations. There were LMW PAH, HMW PAH, and C-PAH in the plots and these pollutants are cause for concern for from public health point of view.

Phenanthrene and 2-Methylnaphthalene (LMW PAH) were found in all the samples at concentration range of 324 µg/kg – 584 µg/kg and 8 µg/kg - 36 µg/kg respectively. Naphthalene is a respiratory toxicnt and a possible human carcinogen (NTP, 2000).

Naphthalene and other substituted naphthalene are very lethal to pulmonary tissues in several organisms (Baldwin *et al*., 2004) and cause olfactory tumor in rats (NTP, 2000). High concentration of NAP and PHE in the plots could be attributed to spillage and incomplete combustion of petroleum products (Prince and Jaylock, 2008). The highest concentration of PAh was recorded in Plot 2 (Maje), a community known for long standing queues of petroleum trucks and mechanics workshops and black (illegal) market petroleum stations.

The HMW PAHs (PYR, BbF, CHR, InP, DBA, BgP) identified in the study had both C-PAH and non C – PAH. The highest concentrations of the HMW PAHs were recorded in DBA and BbF. Ellanhom *et al*. (1997) reported that BbF and DBA are toxic pollutants implicated in human cancer.

Though the total PAH concentrations obtained in this study were lower than the recommended levels of 1000 µg/kg,1500 µg/kg and 5mg/kg from giudlines for cleanups in Denmark, Netherlands and Australia respectively (ANZECC, 1992; MHSPEN, 2000; DEPA, 2002) the values obtained showed slightly to moderate pollution of the plots by these pollutants and called for constant assessment of the study area. Findings by Muze *et al.* (2020) reported a lower PAH concentration in auto-mechanic workshops while Wang *et al.* (2020) reported a very high PAH concentration > 13000 µg/kg in petroleum contaminated area of Loess Plateau, China. Studies from Ogoko *et al*. (2014); Adeyi and Oyeleke (2017) also reported concentrations of soil PAH above the permissible limits in soil. The statistical analysis revealed that there were significant differences in the concentrations of PAH in the various plots suggesting variations in the sources and causes of pollution in the various plots.

This study revealed that 12 out of the 16 priority PAH pollutants by USEPA were detected in the water samples while 4 were not detected. The presence of HMW PAH (BaA, CHR, BbF, DBA BkF, InP) in the water samples is of great concern on the public health of the residents of the communities. These HMW PAHs are carcinogenic, mutagenic and possess several other toxic properties lethal to human and other organisms. The PAH contaminated water in some of these plots are used for domestic and agricultural purposes. PAHs have the tendency to reach and localize in several tissues in the body fat and thereafter exert its effects. The presence of PAHs in the study plots could be attributed to mainly to anthropogenic activities in the plots (Ibe *et al*., 2021). There are several petroleum products sales stations in these plots which have been a source of continuous oil spills to the plots.

Benz(a)anthracene which recorded the highest concentration among the PAHs is a well-known toxic, mutagenic and /or carcinogenic pollutant, which is soluble and readily absorbed from the GIT of mammals (Hussein and Mansour, 2016). The LMW PAHs identified in this study are probable human carcinogens (Bjorseth, 1983). They have also considerable toxic effects on microorganisms and humans causing nausea, diarrhea, kidney and liver damage, teratogenic effects, skin inflammation, genotoxic effects among others (Hussein and Mona, 2016).

The concentrations of PAHs in water obtained in this study have elevated values ranging from ND (not detected) to 0.3312 µg/L in the plots. Methyl naphthalene concentration ranged from 0.1209 to 0.209 µg/L with mean concentration of 0.1520 µg/L which is below the permissible limit of 0.2 µg/L (USEPA, 2001), however, the concentration recorded in Plot 2 (0.2092 µg/L) was above the permissible limits indicating that the water is polluted and not suitable for human consumption. The mean

concentration of 2-methylnaphthalene (0.3091 µg/L) was well above the permissible limit and is of public health interest. Though naphthalene (LMW PAH, $C_{10}H_8$) is not classified as human carcinogen, it has significant acute toxicity to microorganisms (Ideriah and Nwinaa-ie, 2015). The mean concentrations of other investigated PAHs were below the permissible limits but their presence in the plots is still of public health concern. Ideriah and Nwinaa-ie (2015) reported a similar PAH concentration to the current study in Betem underground water while higher concentrations of PAH (13.174 – 26.382 mg/L) were reported by Edokpayi et al. (2018) in rivers and waste water effluents. Also, the findings of Adeniyi at al. (2019) reported PAH concentration of 14.91-206.00µg/L in Buffalo River Estuary in South Africa.

Heavy metals, unlike carbon based substances persist for a long time in the environment, since majority of them do not undergo biodegradation, thus their elimination from contaminated environment is difficult and requires tenacious efforts (Kumar and Srikantaswamy, 2014). Heavy metals in the soil may undergo any of the following reactions in the soil: Adsorption, desorption reaction, dissolution, precipitation reaction, mobility via the soil and bio availability (Violante et al., 2010). The mobility of heavy metal in the soil is influenced by both the biotic and the abiotic factors in the environment, such as the nature of the organic and inorganic ligands in the environment, sorbents, and pH (Violante et al., 2010). The mobility of heavy metals in the environment results in contamination of ground water and their accumulation in plants, which ultimately results in the accumulation of heavy metals in food chain.

Previous studies have established the seasonal variation of heavy metals in the soil. In the plots, erosion, leaching, root uptake, and high precipitation could be the key factors which accounted for the reduction in level of heavy metal observed during the rainy

season in addition to microbial adsorption. Similar results were reported by Yao *et al.* (2014), who observed that rainfall had the tendency to dilute the concentration of heavy metal in the surrounding during the rainy season and spread it to surrounding and even distant environments. The poor road conditions in petroleum products depot, Suleja, Nigeria contributed to increased spills of petroleum products with corresponding increase in heavy metals in the plots. The increased concentration in the level of heavy metals observed during the dry season may be due to high evaporation level during the dry season. Yao *et al.* (2014) also reported a similar increase in heavy metal concentrations due to high evaporation levels which made soil solutions more concentrated.

The Environmental Protection Agency (EPA) recommended limit for Chromium in potable water to be 100 µg/l (Hilgenkamp, 2006; WHO, 2011b). Dagwe (Plot 4), with 1000 meters away from the depot had the highest concentration of Cr in its water bodies during the rainy season, with a calculated value of 0.63 mL/l. This is higher than the WHO maximum admissible limit of Cr in potable water. For all potable water, the WHO admissible limit of Cr is 50 µg/l (Hilgenkamp, 2006; WHO, 2011b). Both PPMC depot water and the Dagwe water sources with respective concentrations of 0.53 mL/l and 0.63 mL/l of Cr are above the permissible level. However, this result is consistent with the result published by Raji *et al*. (2010), who recorded Cr level ranging from 0.510 to 0.800 mg/l in drinking water at Sokoto, Nigeria. These ranges are above the permissible limit set by WHO. Pandey *et al.* (2010) however, was able to obtain lower level of Cr concentration in a river water body. Wogu and Okaka (2011) also recorded lower level of Cr in their study. The Cr concentration obtained in their studies ranged from 0.000 to 0.060 mg/l. Location of water bodies, seasonal variations are some of the

factors which could impact the level of Cr in a water body (Adeleken and Abegunde, 2011).

The recommended limit for Chromium in soil samples is 300 mg/kg (Kinuthia *et al*., 2020). Dikko, which is 500 m away from the PPMC depot, had the highest concentration of Chromium during the dry season, with a calculated mean of 0.68mk/kg, which is within the permissible limit set by WHO. Cr concentrations in the soil samples were higher during rainy season. The Cr in the samples could be attributed to the sources such as sewage discharge from the depot tank farms, which may have reached other areas via leachates and erosion. High doses of chromium can easily enter agricultural soil and subsequently into the body via food chain where it can lead to liver and kidney damages and other potential health hazards (Toth *et al*., 2016; He *et al*., 2019)**.** Similar result was obtained by Adeleken and Abegunde (2011), who reported the level of Cr in soil samples from automobile mechanic village in Oyo State, Nigeria. The level of Cr reported in their studies ranged from 2.0 to 29.75 mg/l, a range that is within the permissible limits set by the United Kingdom.

Cadmium (Cd) is a toxic heavy metal which often produces renal dysfunction and disorder in calcium metabolism. Cd toxicity has also been associated with cancer (Selinus and Alloway, 2005). Most of the side effects associated with Cd are centered on kidney dysfunctions. When ingested, Cd may replace zinc biochemically to cause kidney damage and high blood pressure (Mehbrahtu and Zerabruk, 2011). The permissible level of Cd allowed in drinking by the World Health Organization is 0.003 mg/l (WHO, 1992; Monudu *et al.,* 2010). The Cd values in the analyzed water samples for the rainy season ranged from 0.23 to 1.25 mL/l. These values are beyond the permissible concentration of Cd in drinking water.

Lead exhibit numerous biochemical dysfunctions, it may lead to the inactivation of certain enzymes in the body, interfere the incorporation of calcium into the bones, obstruct nerve transmission and retard brain development (Miheso, 2013). Though several heavy metals are of immense bio-importance to man and other organisms, lead has no known bio – importance in animal physiology and biochemistry thus, accumulation of Pb even in very little concentration may be very lethal (Singh and Kalamdhad, 2011).

The admissible level of Pb in water is 0.01 mg/l (Miheso, 2013). In this research, only Dikko had content below the recommended limits. All the other locations are above the recommended limits both during the rainy and dry seasons. Similar results were obtained by Mebrahtu and Zerabruk (2011), who observed lead content of some water bodies to be as high as 1.347 mg/l.

The maximum concentration of Pb allowed in the soil in both USA and UK are between 100 mg/kg and 200 mg/kg (Mamtaz and Chowdhury, 2006). In this research, the mean values for the concentration of Pb in the soil both during the rainy and dry seasons are well below the permissible limits; however, the Pb concentration was higher during the rainy season. A study by Adeyi and Oyeleke (2017) recorded values of Pb in e- waste contaminated soil in Lagos, Nigeria up to 2,840 mg/kg, which is far above the permissible limits of lead in the soil.

Considered as one of the essential micronutrients, Mn aids the normal functioning of physiological processes in animals and man. However, exposure to higher doses of Mn over a long period may have various side effects which depend on the route of exposure, species, age and sex of the animal (Kohl and Medlar, 2007). The main target organ in which Mn exhibit its toxicity is the nervous system (USEPA, 2014).

The WHO permissible level of Mn in potable water is 0.4 mg/l (Calkins, 2009). In the present study, the mean value of 1.73 mg/l obtained for Mn in all locations is above the permissible level both during the rainy and dry seasons. The concentration of Mn in the water bodies was high during the dry season. This could be attributed to spills from petroleum products and from agricultural applications. Studies by Raji *et al.* (2010), recorded Mn values in water from Sokoto State (Nigeria) ranging from 0.670 mg/l to 0.800 mg/l, which is in agreement with the values obtained in this study. The highest concentration of Mn (3.41mg/kg) is within the permissible limit in soil (WHO, 2003; Canadian Council of Ministers of the Environment, CCME, 2007).

The heavy metals responsible for the rise in the geo-accumulation indices in the sampled plots were in the order; Pb > As >Cr > Cd > Cu > Mn. The Igeo values obtained in the work are similar to the findings of Nwankwoala and Ememu (2018) but are much lower than the values obtained by Adeyi and Oyeleke (2017), who reported high Igeo Pb and Igeo Cu above 6.3 (Extremely polluted) in E-waste soil samples in Lagos and Ibadan, Nigeria. Though findings from Abioye *et al*. (2019) noted some microbial species capable of efficient growth in the presence of some heavy metals, other reports have shown that some heavy metals even in low concentrations have the ability to impair biochemical and physiological activities in microorganisms, plants and animals (Singh and Kalamdhad, 2011).

The high pollution of the plots with the heavy metal (As) could be attributed to atmospheric deposition emanating from activities in the depot in addition to other possible sources like waste from electrical and mechanical workshops and agricultural activities which could have released inorganic arsenic into the plots. Inorganic trivalent arsenic (As [111]) which is very lethal to both human and microorganisms has the potency

to inactivate several enzymes and distort biochemical and physiological activities in the body (IARC, 2012).

The high contamination factor recorded for cadmium in Plot 6 could be as a result of petroleum products spillage often experienced in the plot as well as smelting and mechanic workshops also found in the plot. The pollution load indices showed that the plots were moderately to highly polluted with the metals with Plot 6 recording the highest PLI of 3.60. However, higher PLI was reported by Adeyi and Oyeleke (2017) in e-waste samples in Lagos and Ibadan, Nigeria.

The results obtained for PLI in water were lower than the findings of Adeyi and Oyeleke (2017), who reported CF values of 450 for Pb in e-waste dumpsite in Lagos, Nigeria. The results of PLI obtained in water samples were lower than the findings of Mohd *et al*. (2016), who reported a PLI of 15.75 in heavily contaminated surface water samples.

All the microorganisms (bacteria and fungi) tested for biodegradation of petroleum products in this study were able to utilise hydrocarbons although at different rates. This observation was also made by Ikuesan (2017) where the test isolates utilised hydrocarbons at varying rates. Microbial genera *Bacillus,Pseudomonas,Penicillium* and *Aspergillus* had high degradation capabilities in this study. The capacity to utilise hydrocarbons could be as a result of their tolerance to the toxicity of hydrocarbon, production of hydrocarbon degrading enzymes and also due to their strong physiology to withstand adverse conditions. This result agrees with the findings of Chukwura *et al*., (2016), who also reported high degrading capacity of some fungal isolates from mechanics workshop. Ikuesan (2017) suggested that these differences in degradation rates are associated with the natural ability of the different microorganisms. It may also

be due to the presence of enzymes and biosurfactants, and petroleum products constituents (Jain *et al*., 2011; Oyeleke *et al*., 2017; Karlapudi *et al*., 2018).

The mean viable cell count (VCC) of bacteria and fungi in MSM amended with crude oil generally showed a progressive growth of microbial cells till day 15 across all bacterial and fungal isolates. However, the microbial cells began to decrease after day 15, particularly the cells of *Bacillus thurengiensis* strain M43, *Penicillium notatum* strain P72-30 and *Aspergillus niger* strain ATCC 1015 with the exception of cells of *Pseudomonas aeruginosa* strain PA14, which increased till day 18 before it declined. The general progressive increase of microbial cells observed at the initial and later stages as well as gradual decline of microbial cells depicts a typical microbial growth curve. The reduction in the number of cells occurred as a result of cell aging as well as the exhaustion of nutrients in the mineral salt medium used. As microbial cells grow, they metabolize and release their products into the environment in which they live. Since the medium used in this study is typical of a batch culture, which means that there is no renewal of nutrients and no removal of toxic metabolites, which could eventually deter the growth of the microorganisms, thus, a decline in the number of cells was observed in this study and is in concordance with the report of Nwakanma *et al.* (2016). The catabolic capabilities of these strains to utilise the hydrocarbons as indicated in their growth profiles could be compared to the findings of Sebiomo *et al*., (2010); Ekanem and Ogunjobi (2017) which could be attributed to adaptations of these strains and assimilation ability arising from previous exposures to petroleum products in the environment. Liyanage and Manage (2016), however observed a small increase in bacterial cell counts in an oil bioremediation process by *Bacillus subtilis*.

The mean concentration of 3173.77 mg/kg observed in this study in soil samples was higher than the acceptable upper limit of 2000 mg/kg set by Department of Petroleum Resources, Nigeria (DPR, 2011). Statistical analysis revealed that there were significant differences (P<0.05) in the TPH concentrations in the various plots. Total petroleum hydrocarbon ranks very high among the environmental contaminants and are toxic not only to humans but to microorganisms and other environmental receptors (Olayinka *et al*., 2020). Though TPHs may not be an overall or precise indicator of hazard to humans in the environment (ATSDR, 1999), their presence in the environment however, tells the quality of soil and water bodies and are often used for tracking of source contaminants in soil and coastal waters (Zrafi *et al*., 2013). TPH possess degradation challenges to microorganisms and persists for a long time in the soil hence the results of this current study equally indicate that there could be alterations in the indigenous microbial quality and quantity in the plots. Amadi *et al*. (1993); Osuji and Achugasim, (2006) also reported that high TPH in the soil leads to changes in the physicochemical properties, oxygen deprivation and reduction in gaseous diffusion by the surface oil with corresponding adverse effects on the microbial population which results in poor soil fertility.

The results of this study also noted that numbers of odd and even numbered hydrocarbons in the plots were relatively equal which suggest anthropogenic derived hydrocarbons in the plots. This is in agreement with the findings of Sakari *et al*. (2012) who worked on urban effluent discharge into rivers. The increased number of C16, C18 and C20 in the results also suggested that contamination source could be oil spillage probably from tanks, trucks, workshops or petroleum stations within the plots and this agrees with the findings of Kvenvolden and Cooper, (2003) who worked on natural

seepage of crude oil. The soil samples revealed elevated concentrations of TPHs when compared with the control sample which had TPH concentration within the permissible limits. Results of this study are similar to the findings of Sarki *et al*. (2007); Iwuegbue *et al*. (2007) but lower than the results obtained by Chukwujindu *et al*. (2008); Ogoko, (2014).

The total mean concentration of TPH (223.50 µg//L) observed in water samples in this study was slightly lower than the acceptable standard limit of hydrocarbon in surface and ground water (Sciortino and Ravikumar, 1990; DPR, 2002). The TPH concentrations in Plots 3 and 6 however, were above the EUEPA acceptable limits of 300µg//L in surface water (Sciortino and Ravikumar, 1990), indicating that these plots had high anthropogenic activities and were polluted with TPH. There were significant differences (P<0.05) in the TPH concentrations in the water samples in the various plots. The abundance of n-alkanes in the sample in all the plots suggests anthropogenic source of TPH into the plots (Table 4.38). Among the sampling sites, Plots 3 had the highest TPH concentration because it harbours the petroleum depot and also serves as garage for loaded and waiting loading trucks as well as trucks waiting for petroleum analysis. The TPH concentration obtained in this research is lower than the findings of Alinnor and Nwachukwu (2013); Olayinka *et al*. (2020) but similar to the results obtained by Adeniji *et al*., (2019). The abundance of lower molecular hydrocarbons over the higher molecular hydrocarbons as observed in this study was also reported by Cortes *et al*., (2012), which suggested recent and continuous contamination in the plots.

The presence of PAHs in the environment is of great concern particularly when they occur in concentrations above the permissible limits set by regulatory agencies. Several bacteria and fungi that utilize PAHs have been isolated (White rot fungi, *Ganoderma*

*lucidum*strain CCG1, *Rhodococcus* sp*., Alcaligenes denitrificans, Pseudomonas* sp*., Mycobacterium* sp.) (Agrawal *et al*., 2018). Huge numbers of bacterial and fungal species can mineralize certain PAHs completely to metabolic intermediates, CO_2 and water (da Silva*et al*., 2003). However, the ability of some microbial species to degrade these recalcitrant and lethal contaminants (PAHs) in the current study raises hope for the continual sustenance of the ecosystem in that environment.

The degradation analysis of the 16 priority PAH pollutants by four screened bacterial and fungal isolates in the current study showed that *Pseudomonas aeruginosa* strain PA14 had the maximum total PAHs degradation (84.80 %), followed by *Aspergillus niger* strain ATCC 1015 (72.11 %), *Bacillus thuringiensis* strain M43 (65.75 %) and *Penicillium notatum* strain P72-30 (63.77 %). These isolates showed that they are potential candidates for degradation of hydrocarbon polluted environment.

Naphthalene was most mineralized in the medium by *Bacillus thuringiensis* strain M43 followed by *A. niger* strain AtCC 1015*, P. aeruginosa* strain PA14and *P. notatum* strain P72-30 in that order. *Bacillus* sp. strains have been reported to be very efficient in the degradation of hydrocarbons from oil polluted environments (Ijah and Ukpe, 1992; Ijah and Ndana, 2003). Ijah and Antai (2003b) also reported *Bacillus* sp. as the predominant microorganisms in severely polluted soil samples. Pathak *et al*. (2009) reported that *Pseudomonas* sp. HOB1 was highly potent in degrading high concentrations of naphthalene under optimized conditions.

The results of degradation of anthracene (ANT) as determined by GC-MS analysis showed that among the four isolates *P. aeruginosa* strain P A14 showed the highest percentage (77.5 %) utilization while *P. notatum* strain P72-30 had the lowest utilization (64.0 %). The ability of the isolates to utilize ANT could be attributed to production of

enzyme with ability of ANT hydroxylation into anthraquinone and subsequently into dihyroxyanthraqiunone which is less toxic than the parent compound (Guiraud *et al.*, 2008). Brodkorb and Legge (1992) reported a lower percentage (37.7 %) of ANT mineralization in liquid culture of *Phanerochaete chrysosporium* isolated from oil contaminated soil.

All the microbial isolates mineralized completely InP and DBA in the medium. BgP was also mineralized by all the isolates with the exception of *Bacillus thuringiensis* strain M43. *Penicillium notatum* strain P72-30 had the least percentage (56.5 %) degradation of perylene. This may be connected with the degree of tolerance to toxicity of the PAHs in the medium. The findings of Heshma *et al*. (2006) recorded 70 % and 78 % degradation of perylene and Benzo(g,h,i) perylene respectively in a low concentration of the PAHs.

Chrysene (34 %), BbF (46.37 %), and BaP (53.12 %) were least degraded by the microbial isolates. Kotterman *et al*., (1998) reported a complete degradation of Benzo(a)pyrene by White rot fungi. CHR, BbF and BaP are classified as carcinogenic PAHs and have shown stiff resistance in biodegradation. The percentage of priority PAH degraded by the screened isolates in this study is very much encouraging and their potentials can be harnessed for bioremediation of PAH polluted environment.

The primers used for the identification of PAH degrading genes in this study were selected based on their wide coverage of bacterial and fungal catabolic genes. PAHE2 and PAHE1 primers have been recommended to be very effective in amplifying various genes involved in the degradation of PAHs (Cebron *et al*., 2008, Hanif *et al*., 2012). The primers anneal to the DNA fragments and the DNA polymerase synthesizes the remaining segment of the DNA strand. After PCR amplification, there was a marked

increment in the quantity of the DNA as observed when subjected to gel electrophoresis. The two fungal isolates were amplified by PAHE1 primer but could not be amplified by the PAHE2 primer. This showed that the primer sequence coding for the PAH degrading gene was absent in the organism and was not amplified. PAHE2 primer amplified only *Pseudomonas aeruginosa* strain PA14 gene indicating the presence of the sequence coding for the PAH degrading gene in the organism while PAHE1 primer amplified only *Bacillus thuringiensis* strain M43 gene indicating the presence of the sequence coding for the PAH degrading gene in the organism. The results of this research indicated that the four isolates *Bacillus thuringiensis* M43, *Pseudomonas aeruginosa* PA14, *Penicillium notatum* P72-30 and *Aspergillus niger* ATCC 1015 utilized PAH as sole source of carbon and each possessed either of the PAH degrading genes (pahE1 and pahE2).

CHAPTER FIVE

5.0   CONCLUSION AND RECOMMENDATIONS

5.1.1   Conclusion

The microorganisms isolated from soil and water sources in communities around petroleum products depot in Suleja, Nigeria were capable of utilising crude oil, kerosene, diesel and engine oil at varying rates. *Pseudomonas aeruginosa* had highest ability to utilize kerosene while *B. thurengiensis* strain M43 was most efficient in utilizing diesel and engine oil. *Aspergillus niger* strain ATCC 1015 and *Penicillium notatum* P72-30 recorded a consistent increase in viable counts and also exhibited highest capacity to utilise the petroleum products compared with other fungal isolates. Thus, the bacterial species *Pseudomonas aeruginosa* and *Bacillus thurengiensis* as well as fungal species *Aspergillus niger* and *Penicillium notatum*can be effective in the bioremediation of petroleum products contaminated sites. Water sources from the plots were contaminated with coliform bacteria and poses great challenge to public health.

There were considerable amounts of heavy metals in soil and water sources in the community around the petroleum products depot. There were significant differences (P< 0.05) in the concentrations of heavy metals in the various plots, and this could be attributed to different anthropogenic sources in the plots. However, the concentrations of Cr, Mn and Cu in water samples were within the permissible limits by WHO. The concentrations of As, Cd, and Pb were above the acceptable limits in soil and water in most of the plots.

The study revealed pollution of the soil and water samples by PAH contaminants at varying concentrations with more of the LMW-PAHs, indicating fresh pollution from mainly anthropogenic sources. *Bacillus* thuringiensis M43, *Pseudomonas aeruginosa*

PA14, *Penicillium notatum* P72-30 and *Aspergillus niger* ATCC 1015 utilized PAH as sole source of carbon and each possessed either of the PAH degrading genes encoded in pahE1 and pahE2.

5.1.2 Recommendations

It is recommended that:

1. *Bacillus thuringiensis* M43, *P. aeruginosa* PA14, *P. notatum* P72 – 30 and *A. niger* ATCC1015 are suitable for bioremediation of PAH polluted soil.

2. PAH genes (pahE1 and pahE2) can be incorporated into other microorganisms for enhanced bioremediation process.

3. Arsenic, Lead and Cadmium which were in considerable high concentrations in the soil and water sources analysed can be remedied by ecological friendly methods such as biosorption (water and soil) and phytoremediation (soil).

4. There should be periodic assessment of the soil, water and air in the communities around Petroleum Products Marketing Company (PPMC) depot, Suleja, Nigeria, to ensure safety in public health of the communities.

5. Since some of the polluted plots are used for agricultural activities in these communities, agricultural products in these communities should be properly assessed before consumption.

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**Appendix A: Activities around PPMC Depot, Suleja**

(a) Tankers awaiting loading at the Depot (b) Unplanned settlements (Maje) (c) Unplanned settlements (Dikko)(d) Waste oil from a tanker (e) Pollution of the environment by fumes from a tanker (f) Fire incident at Suleja depot (Jan., 2017)

Appendix B: Classification schemes and pollution models for soil and water utilized in this study

Contamination Index (Lacatusu, 2000)		Geoaccumulation Index (Odewande and Abimbola, 2008)		Pollution Load Index (Thomilson et al., 1980)	
Value	Pollution/Contamination level	Value	Pollution level	Value	Pollution level
< 0.1	Very slight contamination	$Igeo \leq 0$	Unpolluted	≤ 0	Background concentration
0.1- 0.25	Slight contamination	$0 < Igeo \leq 1$	Slightly polluted	$0 < PLI \leq 1$	Unpolluted or moderately polluted
0.26-0.50	Moderate contamination	$1 < Igeo \leq 2$	Moderately polluted	$1 < PLI \leq 2$	Moderately polluted
0.51-0.75	Severe contamination	$2 < Igeo \leq 3$	Moderately-severely polluted	$2 < PLI \leq 3$	Moderately to highly polluted
0.76-1.00	Very severe contamination	$3 < Igeo \leq 4$	Severely polluted	$3 < PLI \leq 4$	Highly polluted
1.10-2.00	Slight pollution	$4 < Igeo \leq 5$	Severely extremely polluted	$PLI > 5$	Very highly polluted
2.10-4.00	Moderate pollution	$Igeo > 5$	Extremely polluted		
4.10-8.00	Severe pollution				
8.10-16.00	Very severe pollution				
> 16.00	Excessive pollution				

Appendix C: Chromatographs of PAH in soil and water samples
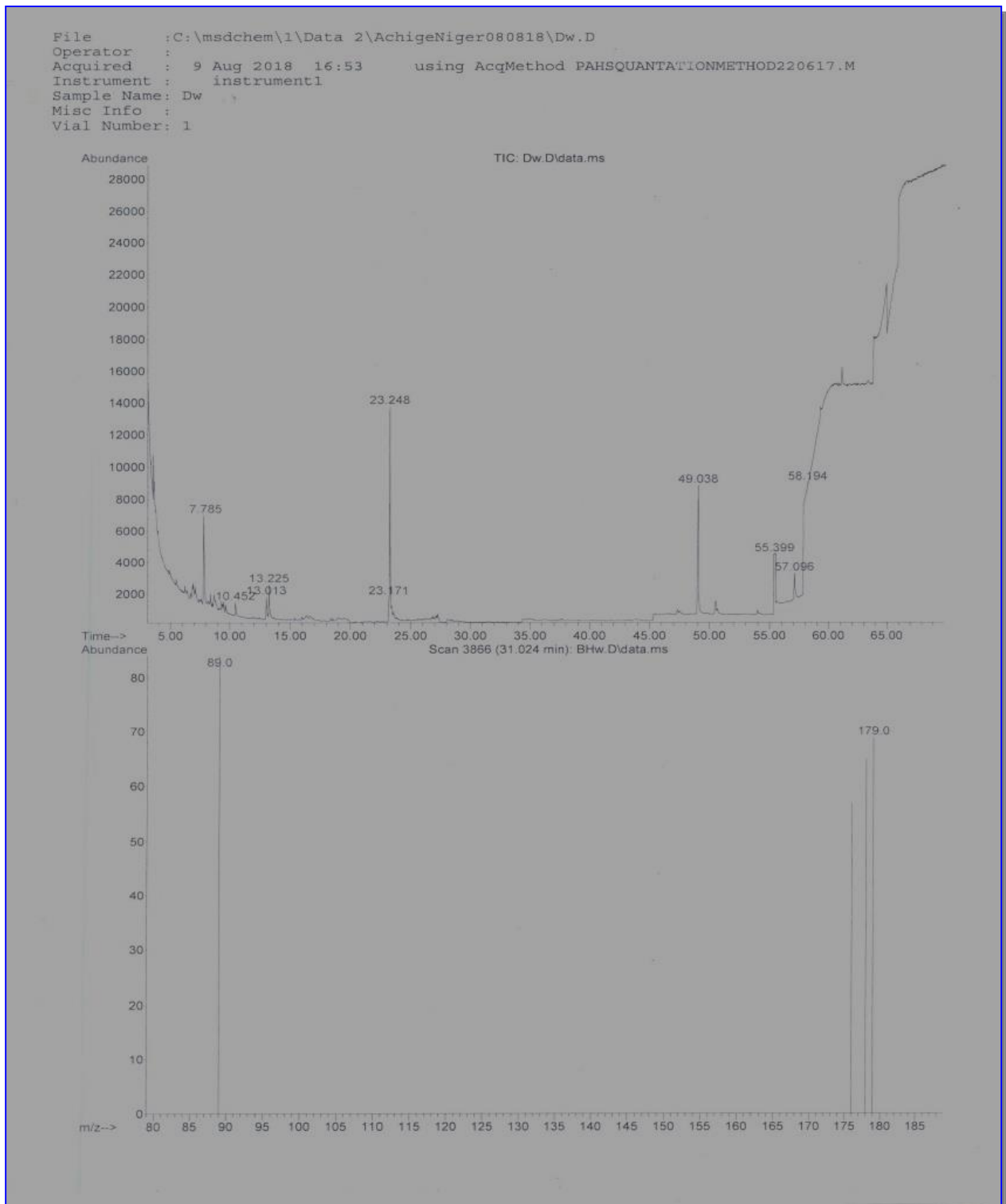
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Instrument :   instrument1
Sample Name: Dw
Misc Info   :
Vial Number: 1

Abundance   TIC: BHw.D\data.ms

30000
28000
26000
24000
22000
20000
18000
16000   23.248   59.274
14000
12000
10000   49.038
 8000   7.786
 6000
 4000
 2000

Time-->   5.00   10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00
Abundance   Scan 3866 (31.024 min): BHw.D\data.ms

        89.0

 80

 70   179.0

 60

 50

 40

 30

 20

 10

  0
m/z-->   80   85   90   95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185

208
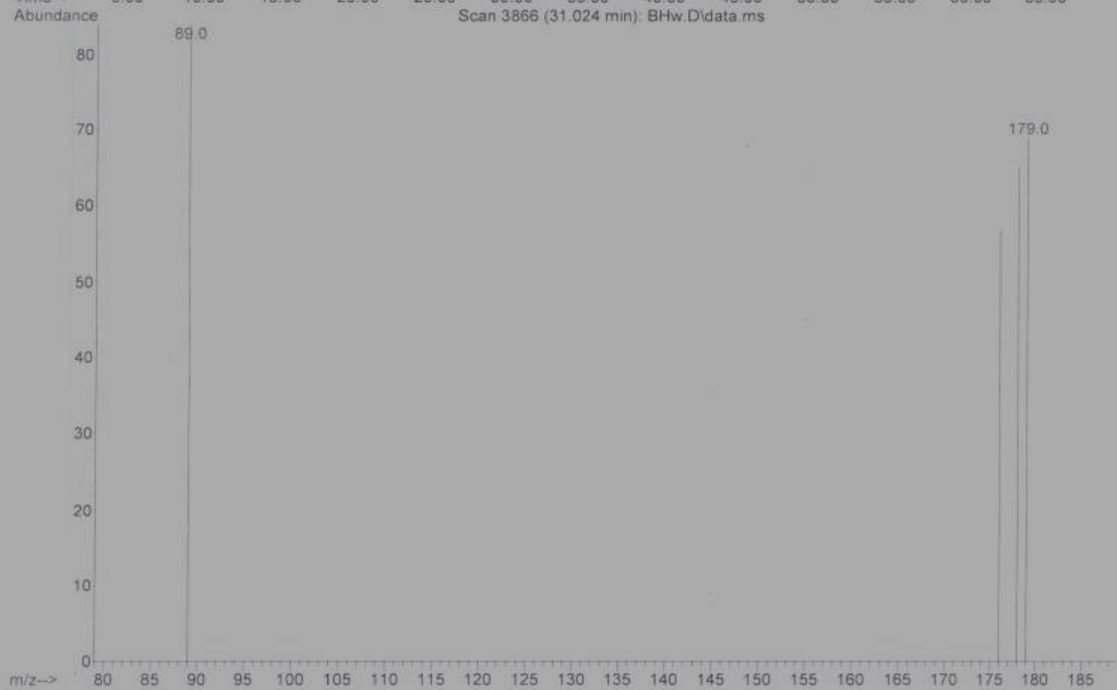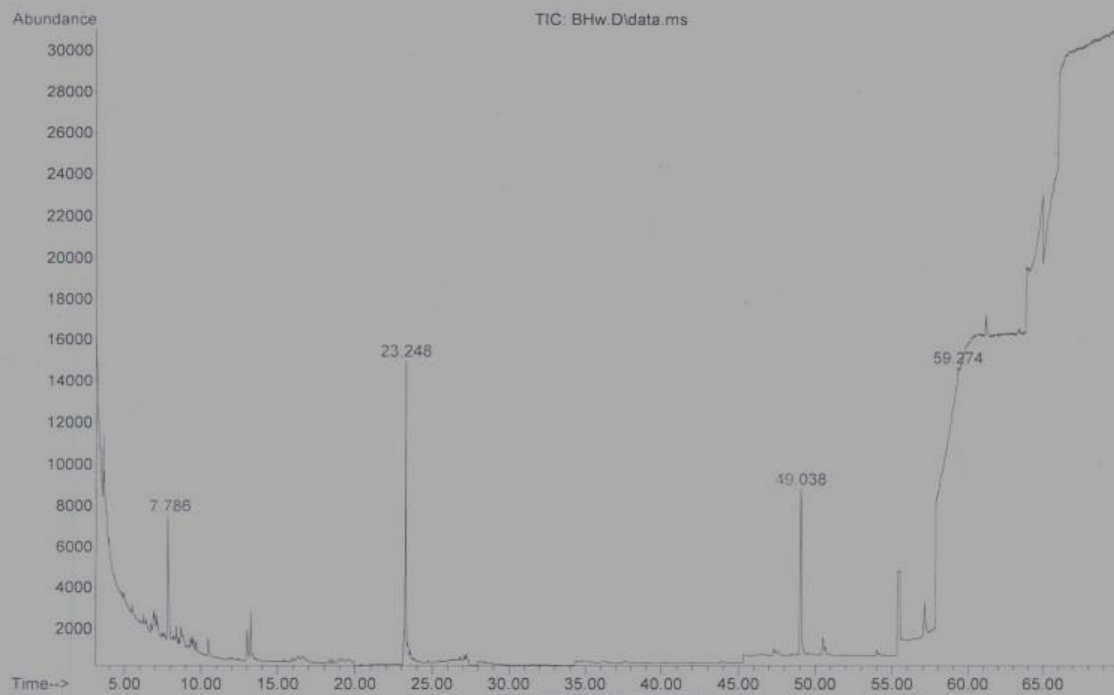
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Instrument :   instrument1
Sample Name: Mw
Misc Info   :
Vial Number: 1

File   :C:\msdchem\1\Data 2\AchigeNiger080818\Depot soil.D
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Misc Info :
Vial Number: 1
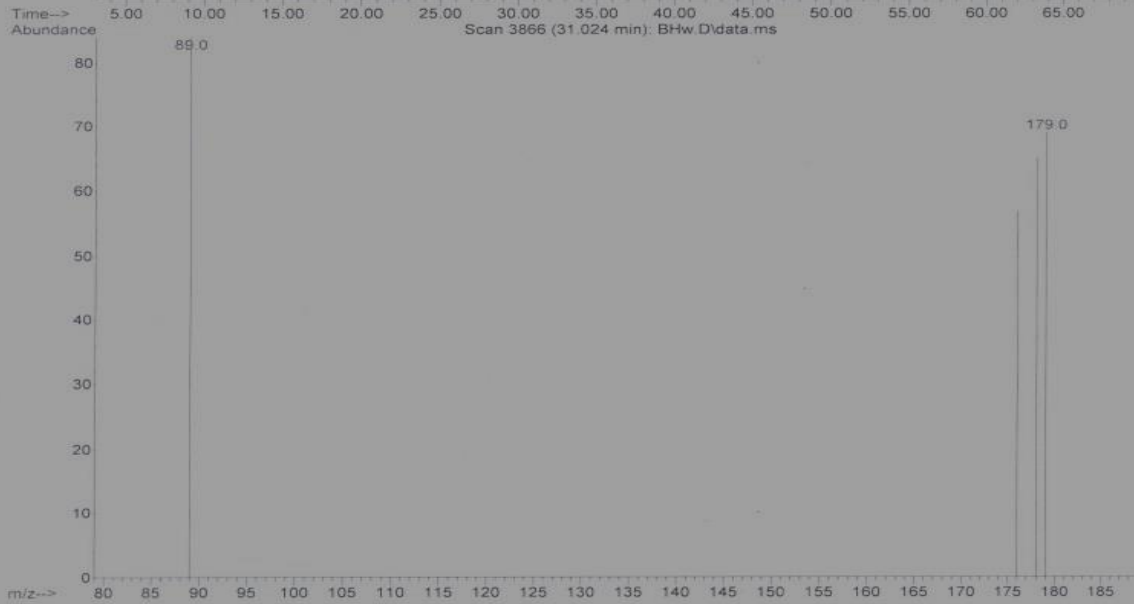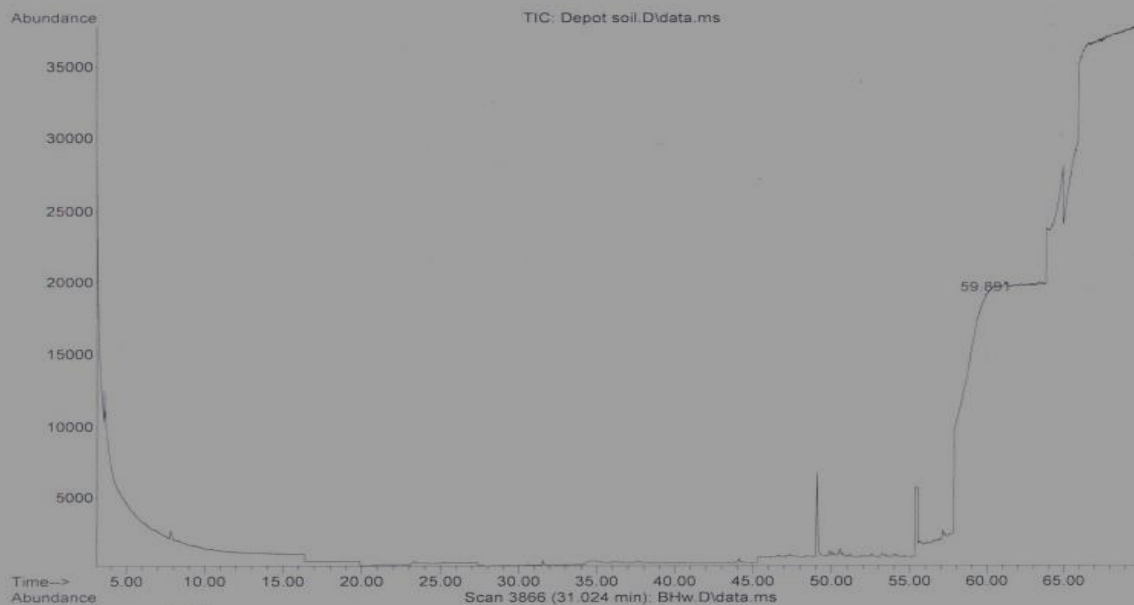
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Vial Number: 1
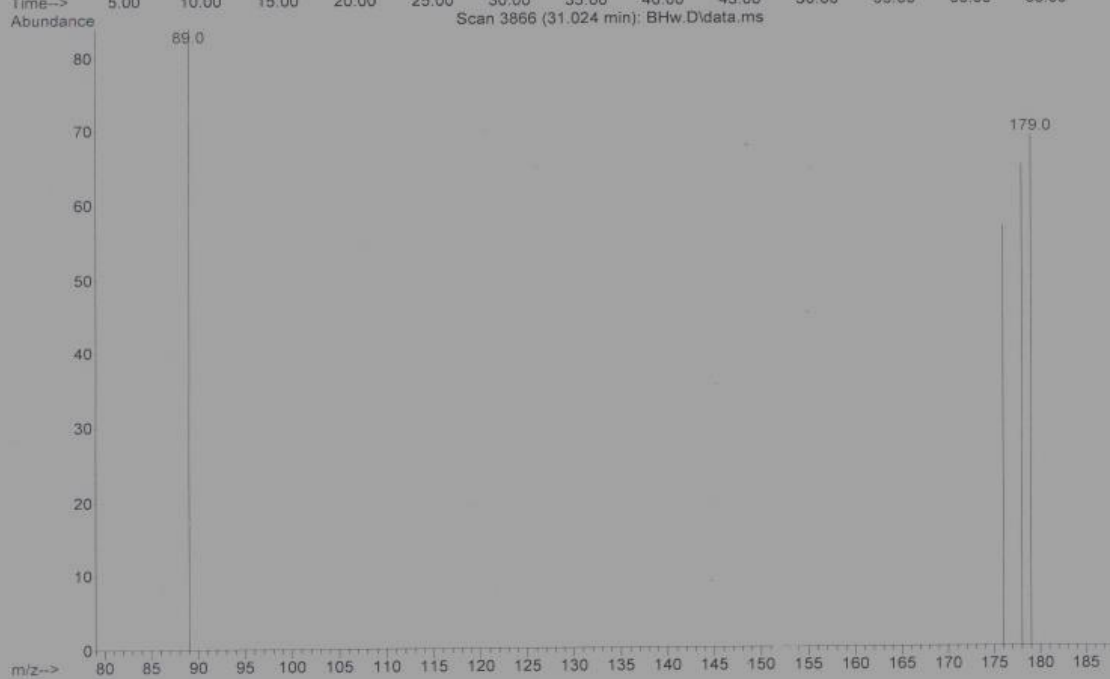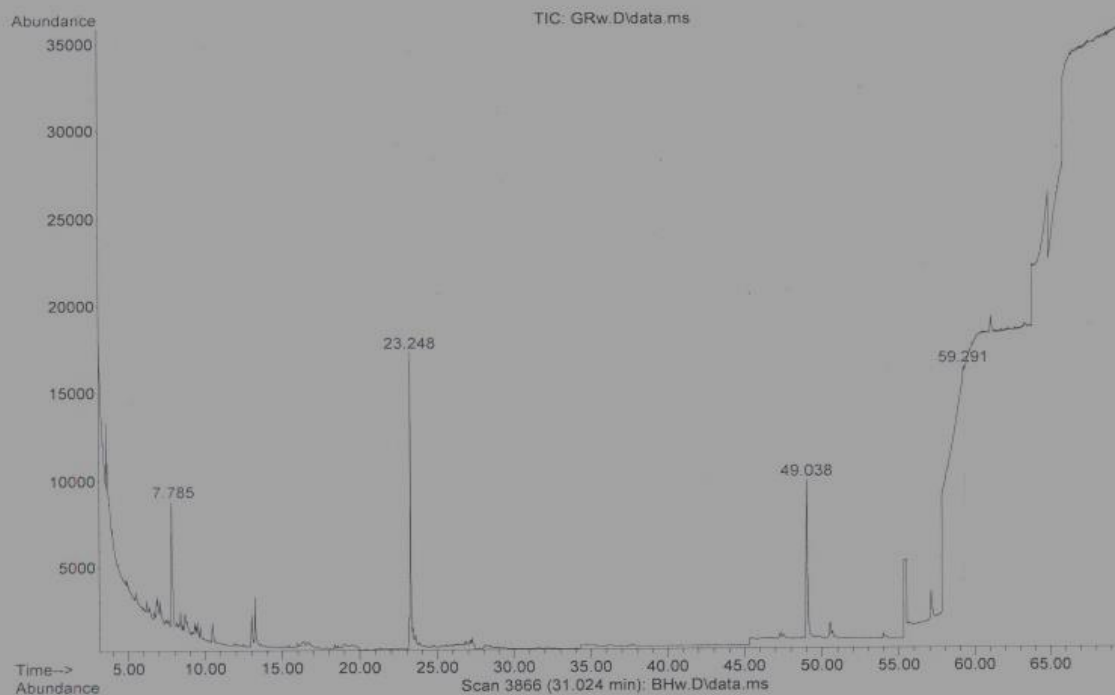
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Operator   :
Acquired   :   8 Aug 2018 12:47   using AcqMethod PAHSQUANTATIONMETHOD220617.M
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Misc Info   :
Vial Number: 1

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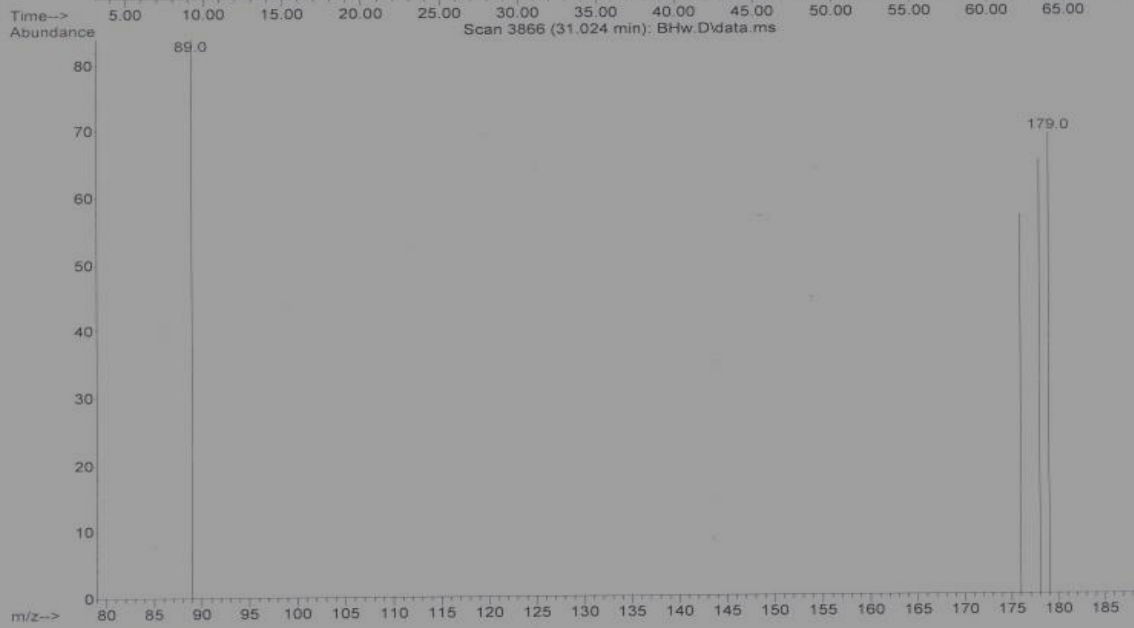
212
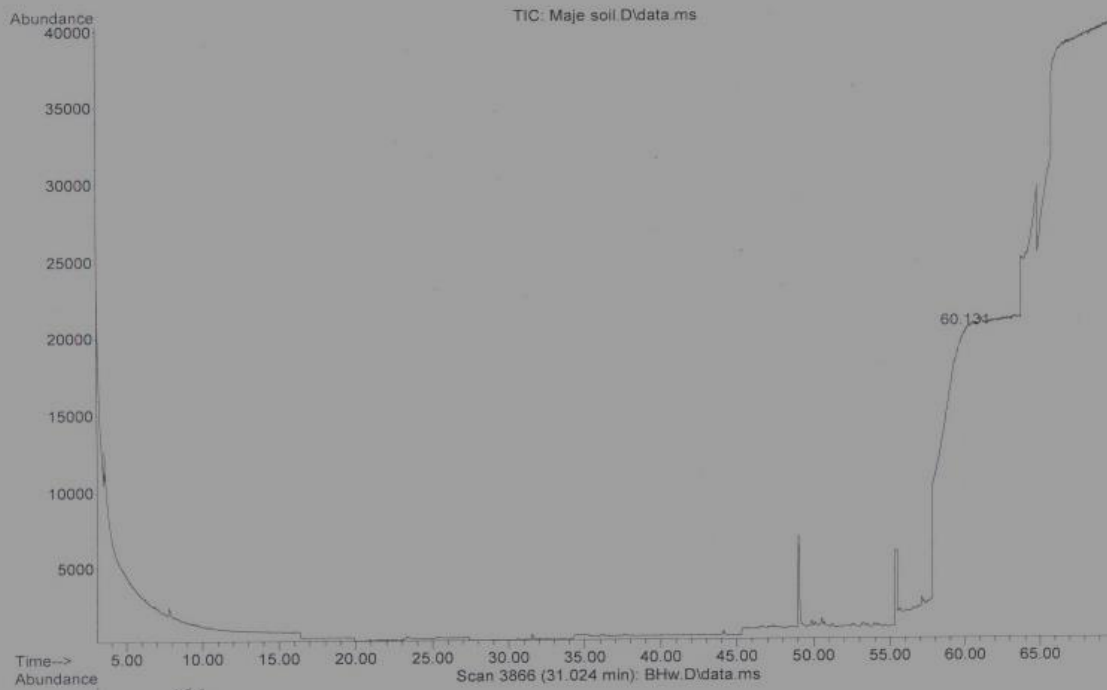
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Operator   :
Acquired   : 10 Aug 2018 11:02   using AcqMethod PAHSQUANTATIONMETHOD220617.M
Instrument :   instrument1
Sample Name: MSw
Misc Info   :
Vial Number: 1

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Acquired   : 8 Aug 2018 15:25   using AcqMethod PAHSQUANTATIONMETHOD220617.M
Instrument :   instrument1
Sample Name: Soil outside depot
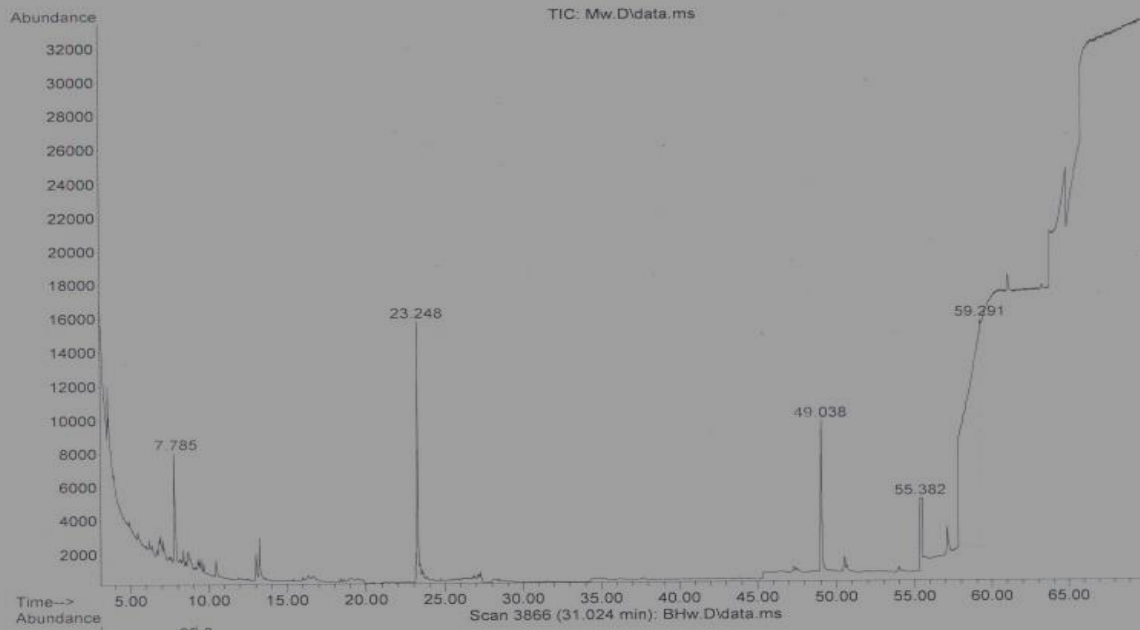Misc Info   :
Vial Number: 1
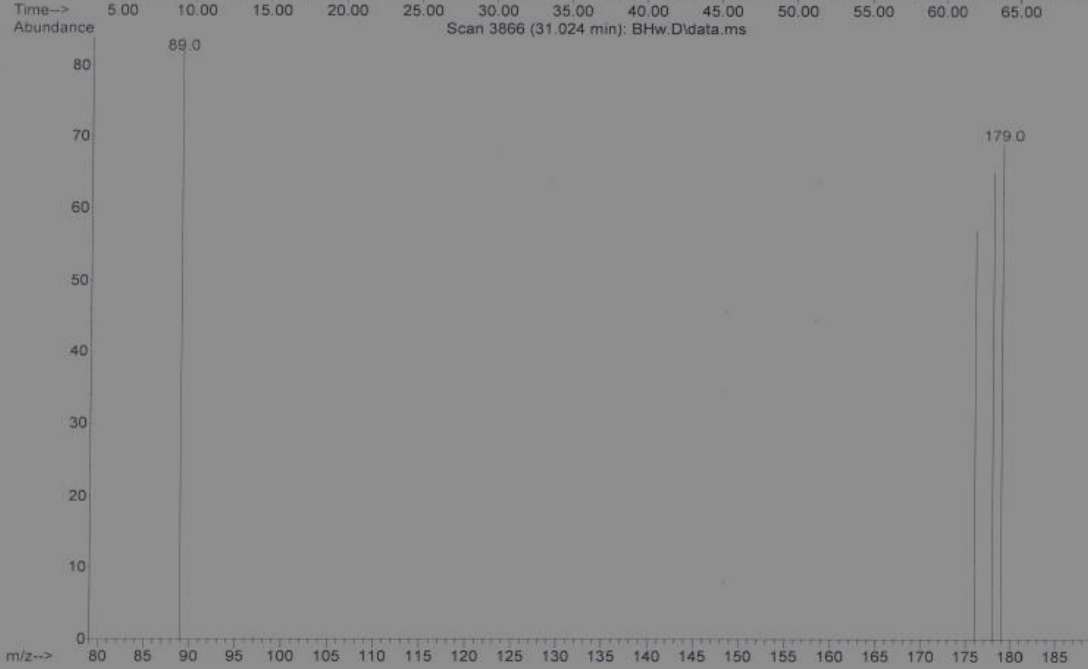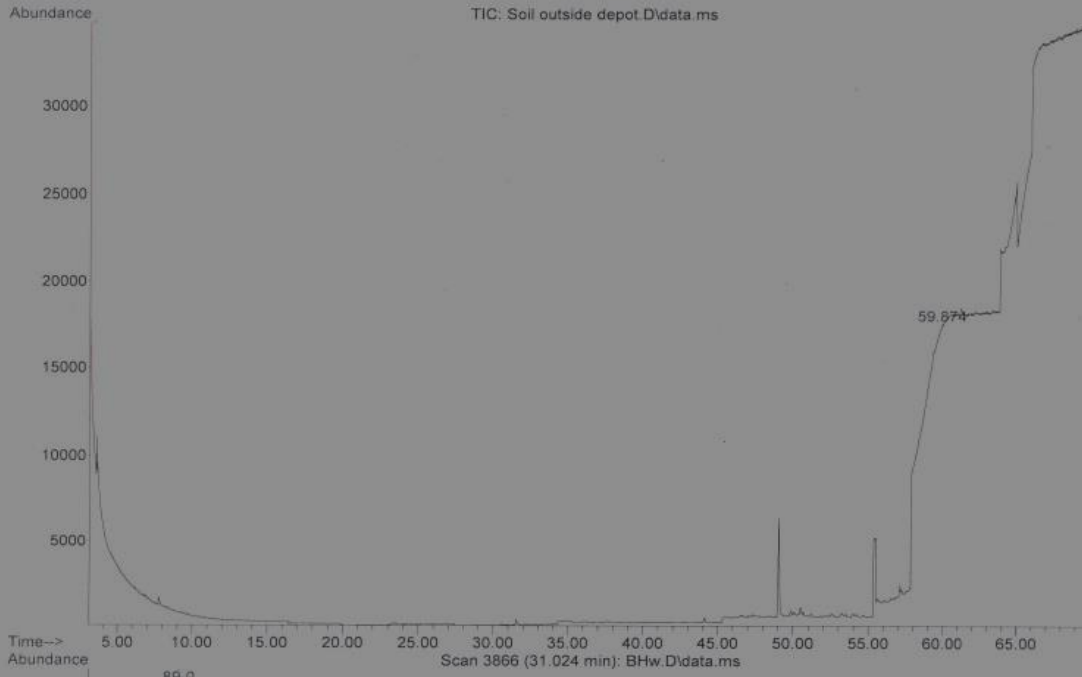
File   :C:\msdchem\1\Data 2\AchigeNiger\Soil-control.D
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Instrument :   instrument1
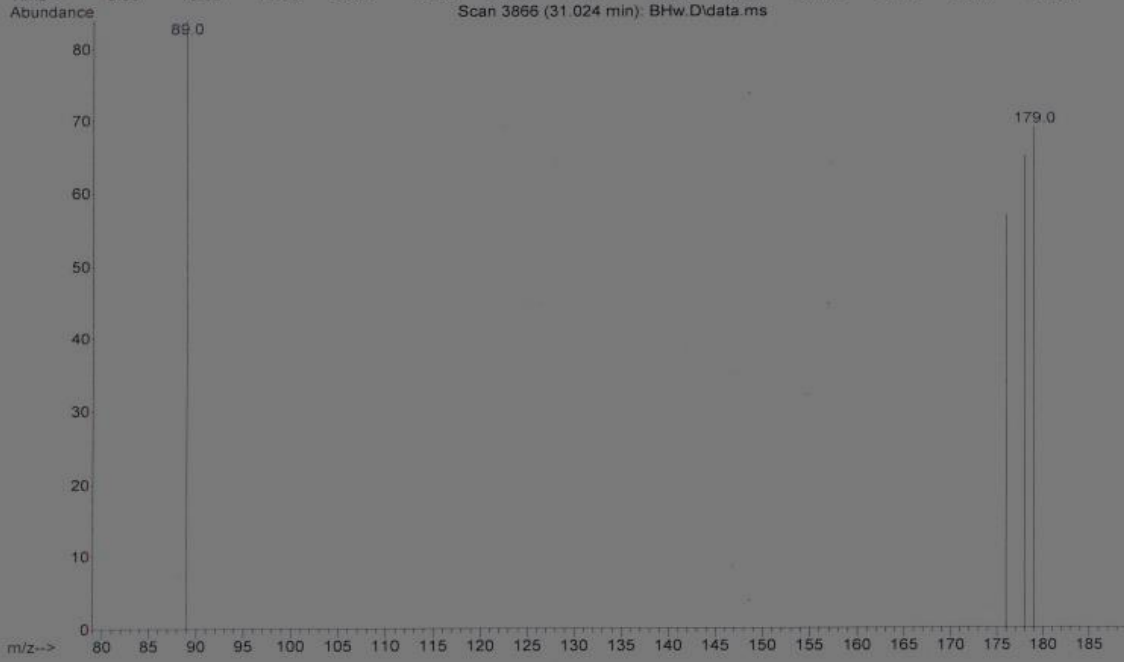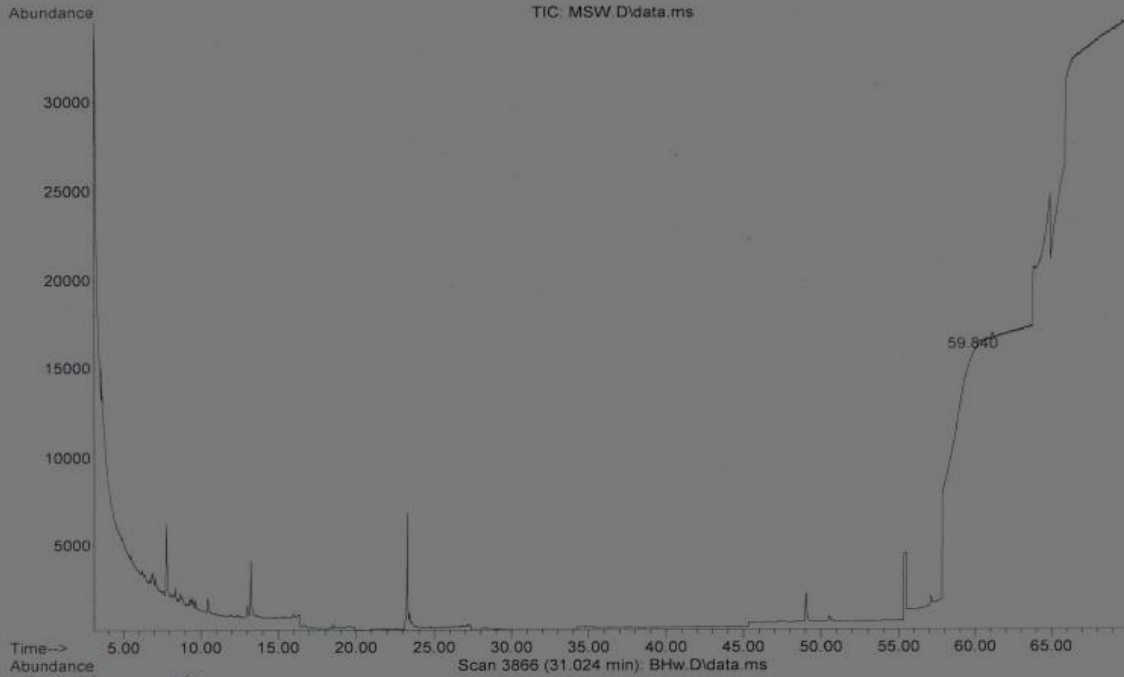Sample Name: Soil-control
Misc Info :
Vial Number: 1

TIC: Soil-control.D\data.ms

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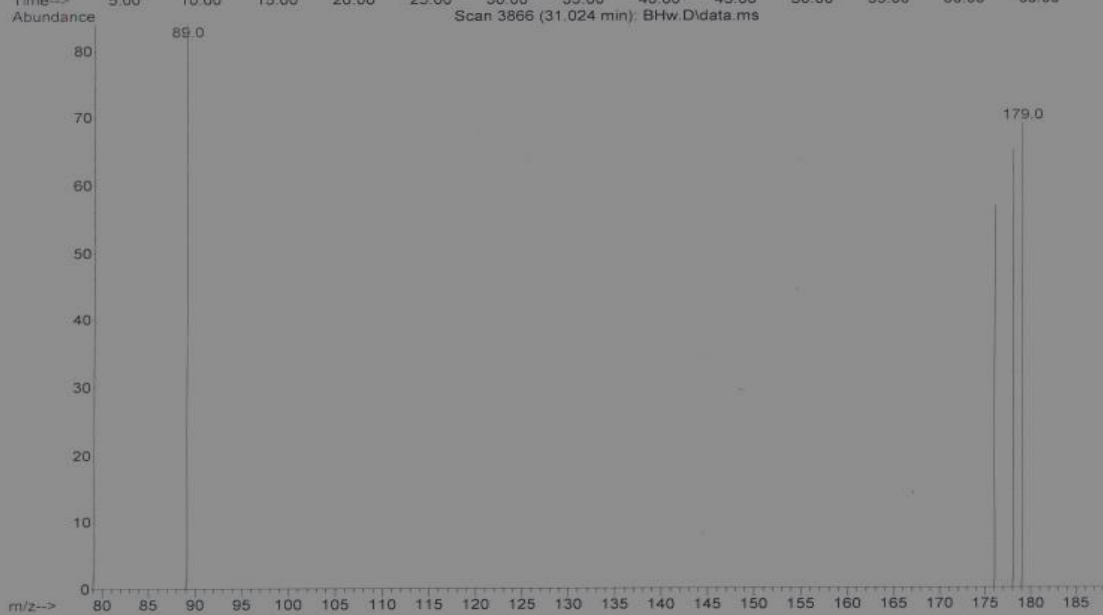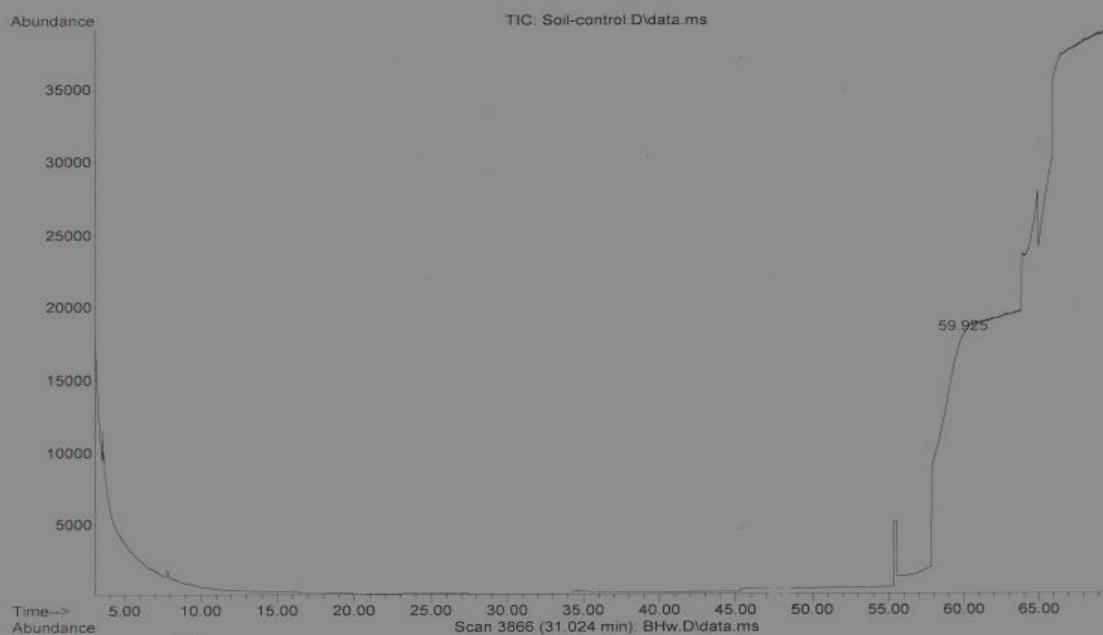
Appendix D: Molecular identification of screened bacterial and fungal isolated used for   PAH degradation

Gene sequence of *Bacillus thuringiensis* strain M43 (98.8 % similarity)

GATTAGTATTGGTCGATCATGGCTCAGGTGCGGTTGGATCACCCCCTTAGAGTT
TGACTGGCTCAGGTGCGTATGGTCCCCCCCTTTAAAGGATGCACGGGGAAACT
ACTGGAAACCGTATGTAATAACGGATTACGTCCTTTGACCAAAGAGGGGGACC
TTCGGGCCTCTTGTCATCAGATGTGCCCTGATCCCATTAGCTATTAGGTGGGGT
AACGGCTCACCTCTCCGACGATCCCTGATTGGTCTGAGAGGATGACCAGCCATC
TGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCGGGATGGGCAATATTGC
AATCTTGGGCGCAAGCCTGATGTCTGATGCAGCGTGCCGAAGAAGTCCTTCGG
GTTGTAAGATCCTTTCAGCGGTTGGAAGGATTGTGTTTAATAACAGCGGAAATT
GACGTTACCCGGGAAGAACACCGGCTGCTCGGTGCCTAAGCCCGGTAATACGG
AGGGACGTACGTCATCGCGAAGACTGGTTTATGCGCACGCAGGAGTCTGTCAG
TCATTGTGAACCGTGGGCTCACCTGGCACTGCTTCGGATTGGAACTGGAGTCTT
GAGACGGGGAGATTCGGGTGCATGTGAGTGCGAAAGTCTGGTGATACGGTAGC
CATGGGCCACTGCTGAAGCCAGGTCTGTATGAGCTGACACAGCGTGATACAGA
TTTGATACTCTGGCGCGACCGTTCGTGCGGTTTCCCTGAGCTTGCTTCTGAGCTA
CAGTGAACACCGCCCGCGACTGGGCGACGTCCAATCATGATGACGAGTGCGGC
GGCGTGACATGGATTATGATGCTCGAGAATCTACAGTATGGTCAAAGATCTCCT
ATGATCGGGCTCGGACTGGAACGGCTCTGCTGCGCAGTCGTGTGGATGTGGTA
AGCCTATCGCAACATATCTTATGCAAGGTCTCTGAATCGGAGGTACGCAGATAT
TCGAAATGGAGAATGCGCTGAGCTCACGCAATAGCTAGGGCTACACTCGCTAG
TGGCTACTGACAGAGCCTGGAGAGCGGTGCATAGCTCACATCATGATCGGCGC
CCTGAGGAGAGGCGATCACAGTACGGTAGTCCGCTGTTTAC

Gene sequence of *Pseudomonasaeruginosa* strain PA14 (99.0 % similarity)

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TACCAGGCGCTACAGGTGAGTAACAAATACCTAATCTGCCCATAAGACTGGGA

TAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTC

GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTA

GCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAG

AGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC

AGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGT

GAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTG

CTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAA

CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTA

TTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACG

GCTCAACCGTGGAGGGTCATTGGAAAACTGGGAGACTTGAGTGCAGAAGAGG

AAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACC

AGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTG

GTGAGCACACAGGATTAGATACCCTGGCTAGTCCACGCCGTCAACGATGAGTG

CTAAGTGTTAGACGGTTTCCGCCCTTTCAGTGCTGAAGTTATCGCATCTATGCC

ACTCCGCCTGCGGAGTACTGACAGCTAAGCTGAAACTTCAAAGGATTGACGGG

GGCCCGTCACAAGCGATGTAAGCATGTAGCTTGCGTCGAGCACGCGAGACTTT

ACGAAGGTCTGACTCCCTCTGAAATCCTAGAGAAGGGCTTCTCATCAGGACGA

AGTGATAGTGGTCCATGCTTGCGTCAGACTTAAGTGTCTAGAAATTGGCTTACT

CCGACAGAGTCTATCCCATG

Gene sequence of *Aspergillus niger* strain ATCC 1015 (99.5 % similarity)

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CGAAATTTTAGAAAAGCAATGCAATTCCAAGAGAGAAACAACGCTCAAACAA

GTATACTTTGGGGGATACCCCAAAGTGCAATGTGCGTTCAAAAACTGATGATTC

ACTTCTGCAATTCACAAGAAATATCGCGTTTCGCTGCGTTCTTCATCGATACGA

GAACCAAGAGATCCATTGTTAAAAGTTTTGATTATTTTGGTTTGGATGGAAGAA

TAATGGTTGGCGGGGTAAATTCACCAATAATTACCATTCCTAATGGACCCTCCG

GCAGTTCAACCAACGGAAACTTGGTAAGAATTTTTACCTTCAAATGAAAGTTTT

GAAGATTAATTCAAAATTTGACTATCAATAAAAATAATTAAATTGTGTTTTGTT

AAACCTCTGGCCCAACCTATCTCTAGGCCAAACCAAAGCAAGAGTTCTGTATC

AAAAAGACACTGTGTGTAAGGTTTTTCGCCGCGCAGTTAAGCGCTGGCAAAAG

AATACTGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTT

TACTTCC

Gene sequence of *Penicillium notatum* strain P72-30(98.9 % similarity)

TAGGTTAATCATCCTCCGGTTTTGGATGGCCCTTGGTTACGATTTTGCTCCCATC

GGGGCTTATTGCGGGTGAAGAGCCTCATACTTTCGAGGACAGGACGGTGCCGC

CGTTTCTCTCGAGGCCCGCCCCCGGGGGGGGCGCGGCCCAACAACCAGCGGGGC

TGGAGGGGAGAAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGG

CGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAG

TTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTT

GAAAGTTTTGACTGATTGGTATCAATCGACTCAGACTGCACGCTTTCAGACAGT

GTTCCATTGGGGTCTCCGGCGGGCGCGGTCCCGGGGGGCAGGCCCCGGGCCGCC

CGAAGGCGGGCCCGCCGAAGCAACAGGGTACGGTAAGCACGGGGTGGGAGGT

TGGGCCCCGAAGGACCCAGCACTCGGTAATGATCCTTCCGCAGGTTCAACCTA

CGGAAACCTTGTTACGATTTTTAATTCACACTCGCTAT

Appendix E: **Total petroleum hydrocarbon present in soil and water in the various plots**

Bar chart showing TPH in soil and TPH in water across plots:

- Plot 1: soil 1407.18, water 191.2
- Plot 2: soil 3153.25, water 188.29
- Plot 3: soil 4808.59, water 352.01
- Plot 4: soil 2593.36, water 68.88
- Plot 5: soil 2879.27, water 144.15
- Plot 6: soil 4137.98, water 325.22
- Ctr: soil 25.82, water 5.54
- Total mean: soil 3173.77, water 223.5

Legend: ■ TPH in soil   ■ TPH in water
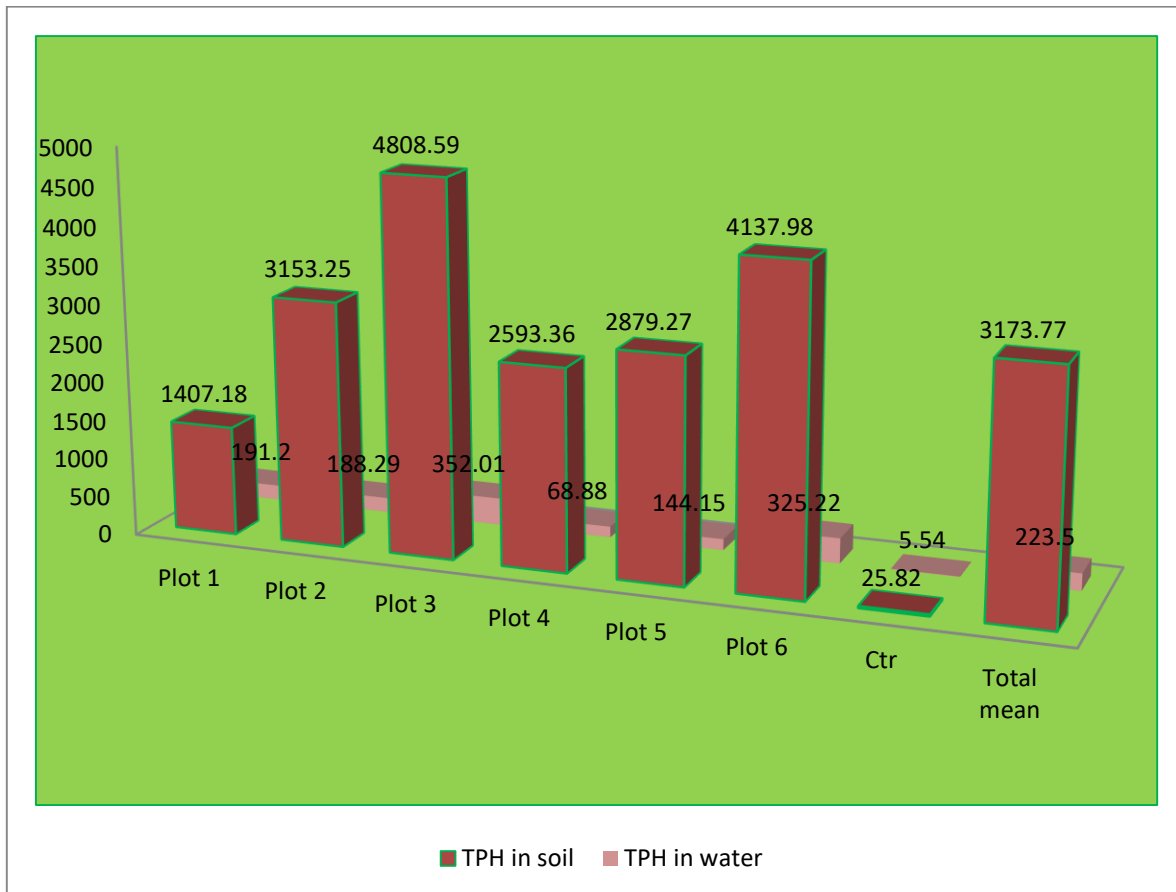
Appendix F: The Principles of Gas Chromatography Mass Spectrometry (GC-MS)

Gas Chromatography(GC) is a technique which works on the principle that a biomolecule will separate into individual substances when heated (Carvalho, 2018). The heated molecules are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the mass spectrometry (MS) unit for quantification and identification. Mass spectrometry identifies compounds by the mass of the analyte molecule. A library of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector

The Process of Gas Chromatography

The components of a sample for gas chromatography are partition between two phases:

1. The stationary phase.

2. The mobile gas phase.

Gas chromatography can be classified into two classes according to the state of the stationary phase. These include gas-solid chromatography (GSC), where the stationary phase is a solid and gas-liquid chromatography (GLC) that uses a liquid as stationary phase. The GLC has greater usage than GSC.

During a GC separation, the sample is vaporized and carried by the mobile gas phase (i.e., the carrier gas) through the column. Separation of the different components is achieved based on their relative vapor pressure and affinities for the stationary phase (Dauenhauer,

2015). The affinity of a substance towards the stationary phase can be described in chemical terms as equilibrium constant called the distribution constant K_c, also known as the partition coefficient, where $[A]_s$ is the concentration of compound A in the stationary phase and $[A]_m$ is the concentration of compound A in the mobile phase.

Kc=[A]s/[A]m (Narayanan, 2011).

The distribution constant (K_c) controls the movement of the different compounds through the column, therefore differences in the distribution constant allow for the chromatographic separation.

Component parts and roles of GC-MS

a. Carrier Gas

The carrier gas plays an important role in the mobile phase in GC-MS technique.It carries the sample molecules along the column while they are not dissolved in or adsorbed on the stationary phase. The carrier gas is inert and does not interact with the sample, and thus GC separation's selectivity can be attributed to the stationary phase alone. However, the choice of carrier gas is important to maintain high efficiency. High purity hydrogen, helium and nitrogen are commonly used for gas chromatography. Also, depending on the type of detector used, different gases are preferred.

b. Injector

This is the place where the sample is volatilized and quantitatively introduced into the carrier gas stream. Basically, a syringe is used for injecting the sample into the injection

port. Samples can be injected manually or automatically with mechanical devices that are often placed on top of the gas chromatograph.

c. Column

The GC-MS column is usually considered as the heart of the GC system, where the separation of sample components takes place. Columns are classified as either packed or capillary columns. The choice of column is mainly dependent on the nature of samples to be analyzed, while packed column is more efficient for larger sample, capillary column is faster and better for complex mixtures.

d. Detector

This identifies molecules by the mass of the analyte molecule. A wide range of known mass spectra covering several thousand compounds is stored on the computer. Mass spectrometry is considered the only definite analytical detector. (Narayanan, 2011)

Appendix G:Principles of Atomic Absorption Spectrophotometry

Spectrophotometry is the interaction between energy and matter. The principle of atomic absorption spectrophotometryis established on the absorption of energy by the atom so that the atom experiences an electronic transition from the ground state to the excited state. In this method, the analysis is based on measuring the intensity of the light absorbed by the atom as the excitation occurs (Narayanan, 2011).

Absorption of atoms required a monochromatic radiation source and a device to evaporate the sample so that the atom is obtained in a ground state of the desired element. Atomic Absorption Spectroscopy (AAS) is a spectroscopy that is based on the absorption of light by atoms. Atoms absorb light at certain wavelengths, depending on the nature of the elements.For example Sodium absorbs at 589 nm, uranium at 358.5 nm while potassium at 766.5 nm (Narayanan, 2011).

The light in this wave has enough energy to change the electronic energy level of an atom. When light with a certain wavelength is passed to a cell that contains the relevant free atoms, some of the light will be absorbed by the atoms in the ground state and will be raised to an excitation level by absorbing more energy. The intensity of absorption is usually directly proportional to the number of metal free atoms in the cell.

The relationship between absorbance and concentration is derived from two laws;

   i.   Lambert's Law which states that: if a monochromatic ray source passes through a transparent medium, the intensity of the transmitted beam decreases with increasing thickness of the absorbing medium.

ii.   Beer's Lawwhich states that: The intensity of the transmitted beam decreases exponentially with increasing concentration of the species that absorbs the beam.

From these two lawsan equation is obtained:

A = Ebc

Where:

E = intensity of the light source

= continued light intensity = molar absorptivity

b = medium length

c = concentration of atoms absorbing light

A = absorbance

From the equation above, it can be concluded that the absorbance of light is directly proportional to the concentration of atoms

Component Parts of Atomic Absorption Spectroscopy

a. Cathode Lamp

Cathode lamps are a source of light in AAS. Cathode lamps have a lifetime or a lifetime of 1000 hours. The cathode lamp for each element to be tested varies depending on the element to be tested, such as the cathode lamp Cu, can only be used for measurement of the Cu element. Cathode lamps are divided into two kinds, namely:

Cathode Lamps Monologam: Used to measure one elementCathode Lamps Multilogam: Used for measurements of some metals at the same time, it's just more expensive.

b. Gas cylinders

The gas cylinder used in AAS is a gas cylinder containing acetylene gas. The acetylene gas in AAS has a temperature range of ± 20,000 K, and there is also a gas cylinder containing N2O gas which is hotter than acetylene gas, with a temperature range of ± 30,000 K.

c. Ducting

Ducting is part of the chimney to suck smoke or residual combustion in AAS, which is directly connected to the outer chimney on the roof of the building, so that the smoke produced by AAS, is not harmful to the surrounding environment. Smoke produced from combustion in AAS, is processed in such a way as in ducting, so that the pollution produced is not dangerous.

d. Compressor

The compressor is a separate device from the main unit, because this tool serves to supply the air needs to be used by AAS, when burning atoms.

e. Burner

The burner is the most important part in the main unit, because the burner serves as a place for mixing acetylene gas, and aqua bides, so that it is mixed evenly, and can burn on the lighters properly and evenly.

f. Exhaust

Disposal on AAS is stored in the drigen and placed separately on the AAS.

g. Monochromator

It functions to isolate one of the resonant lines or radiation from the many spectrums produced by the hollow cathode lamp or to convert polychromatic rays into monochromatic rays as required by the measurement.

g. Detector

Two types of detectors are known, namely the photon detector and the heat detector. Heat detectors are used to measure infrared radiation, including thermocouples and bolometers. The detector functions to measure the intensity of the radiation that is transmitted and has been converted into electrical energy by the photomultiplier. The results of the detector measurements are strengthened and recorded by a recording device in the form of a printer and a number observer. There are two types of detectors as follows:

i. **Light/ Photon Detector**

This detector works based on the photoelectric effect, in which each photon will free electrons (one photon one electron) from materials that are sensitive to light. Photon material can be Si / Ga, Ga / As, Cs / Na.

ii. **Infrared Detector and Heat Detector**

A common infrared detector is a thermocouple. A thermoelectric effect will occur if two metals which have different temperatures are joined together.