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Evaluation of Polycyclic Hydrocarbons Using Thermal Analysis and the Scientific and Biological Study of Isolated Bacteria Isolated from Contaminated Soil in Al-Zeit Street, (AL-Iraq Street), Benghazi, Libya

Iman Nsser Zew *
Department of Occupational Health and Safety, Higher Institute of Engineering Technologies, Benghazi, Libya
*Corresponding author: imanzew288@gmail.com

تحليل ودراسة علمية وبيولوجية للبكتيريا المعزولة من التربة الملوثة في شارع الزيت (شارع العراق)، بنغازي، ليبيا

إيمان ناصر زيو*
قسم الصحة والسلامة المهنية، المعهد العالي للتقنيات الهندسية، بنغازي، ليبيا

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Abstract
In this study, soil samples taken from Al-Zeit Street (AL-Iraq), Benghazi, Libya, are examined for hydrocarbon pollution. Thermo Scientific's TSQ 8000 Evo Triple Quadrupole GC-MS/MS gas chromatography-mass spectrometry was used to analyse soil samples that were randomly selected from the surface, intermediate, and deep deposits. From C12 to C22, thirteen polycyclic aromatic hydrocarbons (PAHs) were found.include(***Acenapthylene, Fluornen, Phenanthrene, Anthrathene, Pyrene, Benzo(a) anthrat, Chrysene, Benzo(b)Flou, Benzo(K)Flour Benzo(a)Pyrene, Indo(1,2,3 cd) P, Dibenzo(a,h)Anth, Benzo(g,h)Penyl).*** and using more sensitive methods like high-performance liquid chromatography or liquid spectral analysis, as well as the linear equation to determine the hydrocarbon contents. This is a connection between the soil, concentration, and analysis (e.g., chromatography's area under the curve). The findings show that hydrocarbon concentrations were below normal, most likely as a result of anaerobic and aerobic bacteria aiding in biodegradation. Concentrations above 50 ng/ml, however, indicate serious contamination and provide hazards to human health and the environment. The pH range of the soil was 8.00 to 9.96. encouraging the growth of microorganisms and the breakdown of hydrocarbons in the presence of nutritional limitations. The effectiveness of biodegradation was decreased by high salinity levels (73.3 to 1196 mg/L), which inhibited microbial enzymatic activity. The study found four bacterial strains that can break down hydrocarbons in anaerobic and aerobic environments: ***Bacillus, Clostridium, Staphylococcus, and Pseudomonas.*** These results demonstrate how bioremediation can be used to reduce petroleum contamination in oxygen-deficient conditions and in the presence of oxygen condition.

Keywords: Hydrocarbon contamination, Diagnosis Of bacteria Bioremediation, Soil pollution, PAHs, Benghazi, Libya.

الملخص

في هذه الدراسة، تم فحص عينات تربة مأخوذة من شارع الزيت (شارع العراق) في مدينة بنغازي، ليبيا، بهدف الكشف عن التلوث بالهيدروكربونات. تم استخدام جهاز كروماتوغرافيا الغاز المقترن بمطياف الكتلة (GC-MS/MS)طراز *Thermo Scientific* من TSQ 8000 *Evo Triple Quadrupole* لتحليل عينات التربة التي جرى اختيارها عشوائيًا من الطبقات السطحية والوسيطة والعميقة. تم الكشف عن ثلاثة عشر من الهيدروكربونات العطرية متعددة الحلقات (PAHs) ضمن نطاق C12 إلىC22 ، شملت: أسينافثيلين، فلورينين، فينانثرين، أنثراسين، بايرين، بنزو(a)أنثراسين، كريسين، بنزو(b)فلورانثين، بنزو(k)فلورانثين، بنزو(a)بايرين، إنديبنو(1,2,3-cd)بايرين، ديبنزو(a,h)أنثراسين، بنزو(g,h)بيريلين. كما تم استخدام طرق تحليلية أكثر حساسية مثل كروماتوغرافيا السائل عالية الأداء (HPLC) أو التحليل الطيفي السائل، إضافة إلى استخدام المعادلات الخطية لتحديد محتوى الهيدروكربونات، لربط العلاقة بين التربة والتركيز ونتائج التحليل (مثل المساحة تحت المنحنى في الكروماتوغرافيا). أظهرت النتائج أن تراكيز الهيدروكربونات كانت أقل من المعدل الطبيعي، ويرجح أن ذلك نتيجة مساهمة البكتيريا الهوائية واللاهوائية في عملية التحلل الحيوي. إلا أن التراكيز التي تجاوزت 50 نانوغرام/مل تشير إلى وجود تلوث خطير يشكل خطرًا على صحة الإنسان والبيئة. تراوح الرقم الهيدروجيني للتربة بين 8.00 و9.96، مما يشجع على نمو الكائنات الدقيقة وتحلل الهيدروكربونات في ظل وجود قيود غذائية. كما أدى ارتفاع مستويات الملوحة (73.3 إلى 1196 ملغ/لتر) إلى تقليل فعالية التحلل الحيوي نتيجة تثبيط النشاط الإنزيمي الميكروبي. وقد كشفت الدراسة عن أربع سلالات بكتيرية قادرة على تحليل الهيدروكربونات في البيئات الهوائية واللاهوائية، وهي*Bacillus* ،*Clostridium* ،*Staphylococcus* و *Pseudomonas*. توضح هذه النتائج إمكانية استخدام المعالجة الحيوية للتقليل من التلوث النفطي في البيئات محدودة الأكسجين وكذلك في البيئات الغنية بالأكسجين.

الكلمات المفتاحية: التلوث بالهيدروكربونات، تشخيص البكتيريا، المعالجة الحيوية، تلوث التربة، الهيدروكربونات العطرية متعددة الحلقات(PAHs) ، بنغازي، ليبيا.

1. INTRODUTION

The environment and human health are at risk when massive amounts of spilt petroleum hydrocarbons build up in the soil. It is commonly known that soils are significant repositories of organic pollutants with a variety of origins and properties **[1],** Large amounts of petroleum oils are used as fuels **[2]** Petroleum hydrocarbons are starting to affect the environment globally. They pose serious health concerns to humans, are poisonous, and are extremely persistent in the environment **[3].** A hydrocarbon's toxicity is also determined by its bioavailability, which is impacted by its chemical and physical properties. The hazards connected to hydrocarbon-contaminated soils can be decreased by using native microorganisms in bioremediation procedures **[4].** Acute impacts of petroleum hydrocarbon product exposure include alterations in the respiratory, visual, and neurological systems. In addition to headaches and migraines, residents of oil-polluted areas also suffer from upper respiratory tract infections, nausea, and irritations of the nose and eyes **[5].** Numerous plant and animal species have gone extinct locally as a result of hydrocarbon and derivative pollution of the environment **[5].** A class of lipophilic organic pollutants, PAHs are produced by incomplete combustion from natural (such as forest fires and bushes) or man-made (such as car emissions, home heating, and cigarette smoke) sources. Due to their high fat solubility, these molecules can quickly disperse throughout the body after being injected. This allows them to pass through cell membranes and settle in adipose tissue, the kidneys, and the liver. Damage to the blood and likely immunosuppression result from exposure to these chemicals. Human cancer can result from long-term exposure to PAHs, and the chemicals' ability to

produce cataracts—that is, changes at the dermal and ocular levels—has been extensively studied exposure can happen by ingestion (from tainted food or air inhalation or drink, or directly, or dermally). PAHs, especially those with three to seven aromatic rings, can induce skin, lung, or gut malignancies, depending on the exposure route. PAHs may cause tumors and mutations in addition to having an impact on the immunological, neurological, and excretory systems **[6].** The high stability, toxicity, and carcinogenicity of polycyclic aromatic hydrocarbons (PAHs) make them extremely dangerous pollutants [7]**.** And cause Genetic mutations, immunotoxicity, teratogenicity, neurotoxicity, strong immune toxicity, chromosomal damage, carcinogenesis, high bioaccumulation potential, and deterioration of ecosystem functioning and treatment of animal and plant life are just a few of the immediate or latent effects that hydrocarbon contaminants can cause **[8]** The ability of microorganisms to transform petroleum hydrocarbon into the necessary carbon and energy is widely known. ***Pseudomonas, Rhodococcus, Acinetobacter, Gordonia, Burkholderia, Enterobacteria, Sphingobium, Novosphingobium, Sphongomonas, Psychrobacillus, and Bacillus*** are a few of the bacteria found in the contaminated area **[8].** In the presence of dissolved oxygen, it was discovered that several bacteria, mostly belonging to the genera Mycobacterium and Pseudomonas, may break down and change polycyclic aromatic hydrocarbons **[9].** the extensive and well-known breakdown of PAHs, such as naphthalene, phenanthrene, acenaphthene, and anthracene, by bacteria through metabolic mechanisms **[10].** The main prerequisite for the degradation by bacteria is simply the presence of dissolved oxygen, which initiates the breakdown of the PAH rings by enzymes. Both aliphatic monooxygenase and aromatic dioxygenase function as catalysts in the oxidation processes in the first phase. In the meantime, the disorder of the hydrocarbons has a significant impact on the collections of microorganisms in soil, which leads to enrichment of the petroleum hydrocarbon selection to the microorganisms. Consequently, the interaction between petroleum hydrocarbon and soil microorganisms and the type of ecosystem found only on biomes shapes the fate of the contaminants according to both their chemical nature and their microbial degradative capabilities. Microbial methods used to monitor the biodegradation of the petroleum hydrocarbon should contain molecular indicators that are chemical, biochemical, and microbiological in order to measure the activity rates of the microorganisms and to achieve the acceptable level of pollution reduction. **[11].** By adding pollutant-degrading microorganisms and increasing their variety, bioaugmentation broadens the scope of complicated pollutants' breakdown **[12].** In order to expedite the elimination of undesirable substances, this approach entails the introduction of native microbes or genetically modified microorganisms to contaminated areas under ideal conditions **[13].** The use of bioaugmentation in soil remediation has been the subject of numerous studies. For example, **[14]** used a bio pile technology system in conjunction with bioaugmentation to study the biodegradation of weathered oil hydrocarbons. **[15]** used cultures supplemented with phenanthrene to extract a Pseudomonas strain (W10) from a diesel-contaminated soil. In the presence of a wide range of hydrocarbons, including aliphatic, monocyclic aromatic, and polycyclic aromatic hydrocarbons, this strain demonstrated the ability to proliferate. This resulted from the strain's capacity to produce a biosurfactant that made hydrocarbons easily accessible for breakdown. Another study **[16]** found that Pseudomonas aeruginosa could break down polycyclic aromatic hydrocarbons (PAHs) such pyrene, fluorene, and phenanthrene as well as n-alkanes (C16 and C19) As the number of benzene rings rises, PAHs become less soluble in aqueous solution, increasing their environmental persistence. A chemical with a high persistence is more likely to cause toxicity. Recently, it has been acknowledged that environmental quality and life quality are related,

Accordingly, the pervasiveness of hydrocarbons in soil, water, air, and sediment poses a major risk to human and environmental health **[17].** as articulated by the World Health Organization **[18].** Low-molecular-weight PAHs include naphthalene, fluorene, anthracene, and phenanthrene, which include two to three benzene rings. High molecular weight PAHs include those that have four or more benzene rings, including pyrene, chrysene, benzo(a)pyrene, and benz(a)anthracene. Higher molecular weight PAHs are liquids or solids at room temperature and frequently exhibit a distinct phase in water, whereas low molecular weight PAHs are gases that tend to leak into the atmosphere **[19].** PAHs sequester in micropores, increasing their resistance to cleanup **[20].** Because of their toxicity and persistence, 13 PAHs have been designated as priority pollutants by the US EPA **Table 2; [21]**. the USEPA designated 13 PAH species as priority control pollutants based on toxicity and frequency (Figure 1) **[22].**

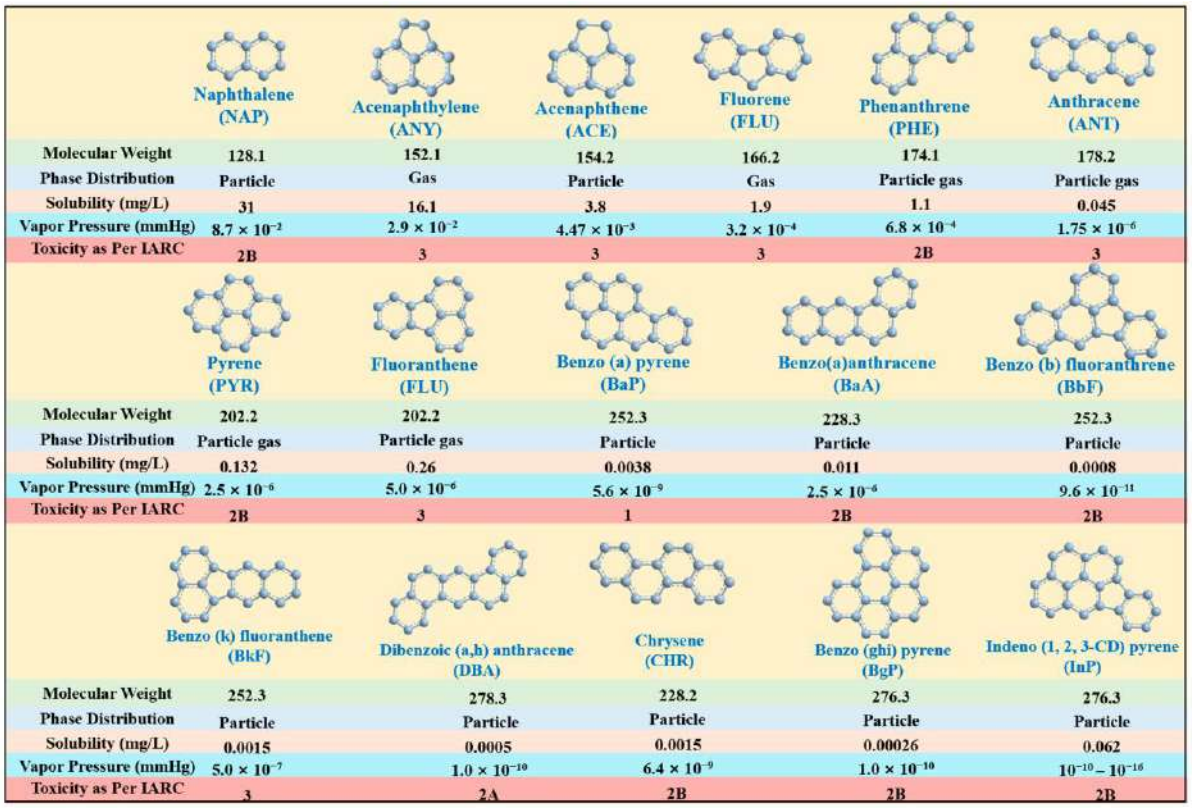
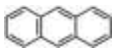
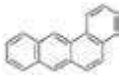
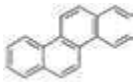
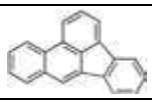
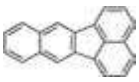
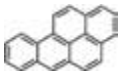
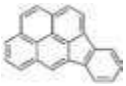
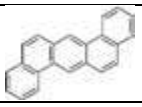


Figure 1: shows the structure, physical characteristics, and toxicity of the major prevalent PAHs.

Table 1: lists certain traits and the PAHs' hazardous classification**.**

PAH compound (s)	MW (g/ mol)	CAS	Molecular formula	Carcinogenic classification	Structure
Acenaphthylene	152	208-96-8	$C_{12}H_8$	Category D: "Not Classifiable as to Human Carcinogenicity" As of right now, there is no proof that it causes human cancer.	
Fluorene	166	86-76-7	$C_{13}H_{10}$	Category D: "Not Classifiable as to Human Carcinogenicity" As of right now, there is no proof that it causes human cancer.	

Phenanthrene	178	85-01-8	$C_{14}H_{10}$	Category D: "Not Classifiable as to Human Carcinogenicity" As of right now, there is no proof that it causes human cancer.	
Anthracene	178	120-12-7	$C_{14}H_{10}$	Category D: "Not Classifiable as to Human Carcinogenicity" As of right now, there is no proof that it causes human cancer.	
Pyrene	202	129-00-0	$C_{16}H_{10}$	Category D: "Not Classifiable as to Human Carcinogenicity" As of right now, there is no proof that it causes human cancer.	
Benzo(a) anthracene	228	56-55-3	$C_{18}H_{12}$	"Probably carcinogenic to humans" is Group 2A's statement. Although there is substantial evidence that it can cause cancer in people, this is not yet proven.	
Chrysene	228	218-01-9	$C_{18}H_{12}$	Group 3: The substance cannot be categorized based on whether it causes cancer in people. This category is most frequently used when there is insufficient evidence of carcinogenicity in people, limited or insufficient evidence of carcinogenicity in experimental animals, and limited or insufficient mechanistic data.	
Benzo(b) fluoranthene	252	205-99-2	$C_{20}H_{12}$	Substances and exposure conditions are classified as category 2A agents.	
Benzo(k) fluoranthene	252	207-08-9	$C_{20}H_{12}$	Substances and exposure conditions are classified as category 2A agents.	
Benzo(a)pyrene	252	50-32-8	$C_{20}H_{12}$	Substances and exposure conditions are classified as category 2A agents.	
Indeno(1 2 3-cd)pyrene	276	193-39-5	$C_{22}H_{12}$	Substances and exposure conditions are classified as category 2A agents.	
Dibenzo(a,h) anthracene	278	53-07-3	$C_{22}H_{14}$	Substances and exposure conditions are classified as category 2A agents.	
Benzo(g,h,i) perylene	276	191-24-2	$C_{22}H_{12}$	Category D: "Not Classifiable as to Human Carcinogenicity" As of right now, there is no proof that it causes human cancer.	

Auher [1] studies Environmental Polycyclic Aromatic Hydrocarbons (PAHs): Health Hazards, Fertility Consequences, and Occupational Exposure in the 2025 year. **Auher [2]** study Bacterial biodegradation of oil-contaminated soil for pollutant abatement contributing to achieve sustainable development goals: A comprehensive review in the 2024 year. **Auher [3]** The bacterial insulation that predominates in the contaminated soil is one of the elements influencing the biological breakdown of oil hydrocarbons.in the 2014 year, **Auher [4]** Access the essential procedure for eliminating some contaminants from the soil in the 2022 year. **Auher [5]** Examine soil recurrence with multi-episode aromatic hydrocarbons analytically in

2021.**Auther [6]** GC MS/MS methods for ambient air pm polycyclic aromatic hydrocarbons analysis in the 2023, **Auher [7]** work by GC/MS Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) Using EPA 8270E in the year 2011.**Auther [8]** Examine how well isolated bacteria from soil contaminated with oil compounds decompose hydrocarbons in 2015. **Auher [9]** reached to The Impact of Hydrocarbon Pollution on the Environment in the 2005 year, **Auher [10]** Using gas chromatography and mass spectrometric detection, dichloromethane extraction was used to identify polycyclic aromatic hydrocarbons in soil (2003) in the 2018 year. **Auher [11]** The examination of petroleum hydrocarbons in soils from different development locations in the 2019 year. **Auher [12]** investigate the biological investigation of raw oil that has been transformed by isolated bacterial fibers from the Tobruk, Libya fire.

2.Object: Analyze the physicochemical properties of contaminated soil samples. And also identify and classify the types of bacteria that degrade hydrocarbons. And their concentrations should be compared to standard levels in order to assess the level of contamination. And examine the potential for using native bacteria for bioremediation and lessen soil-destroying effect of urban sprawl.

3.Search issue: The issue of hydrocarbon-based soil contamination on Benghazi's oil street is a prevalent one that most regions near petroleum products and both public and private fuel stations face.

The quantities of polycyclic aromatic hydrocarbons are lower than the GC-MS-measured values of solution standards., which may contain petroleum hydrocarbons,metels,neutrally occurring radioactive materials,salts,and toxic chemical has the potential to cause contaminated soil and inhibit plant growth.as shown in figure (2).

Figure 2: shows the Benghazi area's soil hydrocarbon contamination samples and issues.

4.Formulation of the problem: These research help determine the most efficient ways to use bacteria in pollution remediation by comparing the efficiency of bacterial species in the analysis of aromatic hydrocarbons. Addressing the variations in concentrations. A thorough assessment of the environmental dangers posed by aromatic hydrocarbon contamination of the soil.

5.Mythology of the Proposed Solution: Establishing integrated and more comprehensive toxic and tactic to remove multiepitope, the multiepitope through bacteria, using biological motivation, biological, biological deeds, chemical therapy, vegetable deeds or precise organisms to determine and classify the multi-essential hydrocarbons that are toxic and polluted rings of the soil

6. Materials and Methods

6.1 Study Area

Al-Zeit Street (Iraq) in Benghazi, Libya, a neighborhood with a lot of petrol stations and heavy traffic, is where soil samples were taken. As shown in Figure (3)

Figure 3: shows the study site for the samples taken in Al-Zeit Street, Benghazi, Libya.

6.2 Sample Collection and Preparation

Three depths of soil were sampled: deep (30–50 cm), middle (10–30 cm), and surface (0–10 cm). Prior to analysis, the samples were sieved, allowed to air dry, and then stored at 4°C. as shown in table below (2) and figure (4)

Table 2: displays the locations of the nine distinct soil sample collection sites.

Type of Sample	Site (1)	Site (2)	Site (3)
Surfaces	X1	Y1	Z1
intermediate	X2	Y2	Z2
deep	X3	Y3	Z3

Figure 4: shows the samples taken from the soil in Al-Zeit Street**.**

6.3 Hydrocarbon Analysis

Measurements of PAH concentrations were made using gas chromatography-mass spectrometry (GC-MS/MS). Thermo Scientific's TSQ 8000 Evo Triple Quadrupole GC-MS/MS equipment was used to conduct the analysis.

6.4 The Positive

1. **Systematic Comprehensiveness**

- This study collected between chemical analysis (such as GC-MS/MS) and biological study
- (bacteria insulation and activity analysis), which reflects an integrated approach to understanding the recurrence and processing mechanisms

- then identifying 13 types of PAHs multi-episodes and evaluating their concentrations according to environmental standards.

2. Bacterial diversity

Isolated four bacterium strains, such as (***Bacillus** **Pseudomonas** **and* staphylococcus* Clostridium**) being able to analyze hydrocarbons in ***aerobiotic*** and its ***anaerobiotic***, it shows the possibility of using it in vital therapy.

3. Analysis of environmental Factors

The study linked the properties of the soil (such as the acidity PH and Salinity) and the effectiveness of biological decomposition which provides a vision about the gay conditions of bacteria activity.

4. Scientific recommendations

It included clear recommendations, such as the use of local bacteria in vital treatment and periodically monitoring pollution. This reflects applied orientations to protect the environment.

7. Discussion

7.1 Thermo Scientific TSQ 8000 Evo Triple Quadrupole GC-MS/MS Easy operation, brilliant results

For laboratories looking to increase their triple quadrupole GC-MS/MS productivity, the Thermo ScientificTM TSQTM 8000 Evo Triple Quadrupole GC-MS/MS is the perfect choice. It is the most recent iteration of the wildly popular TSQ 8000 GC-MS/MS system, which is the progression of unstoppable productivity, straightforward MS/MS, and ultimate performance SRM. The requirements of high throughput analytical labs were taken into consideration when designing the TSQ 8000 Evo triple quadrupole GC-MS/MS. It is a special system that combines hardware and software capabilities in a way that makes it easy for labs to adjust to their changing surroundings and consistently produce high-quality findings on schedule. Thermo Scientific™ TRACE™ 1300 GC or TRACE 1310 GC, which provide the special flexibility of immediate attach injector, is combined with the mass spectrometer.as well as detector modularity. To achieve the highest level of analytical productivity, add a Thermo Scientific™ AI/AS 1310 auto-sampler for automated liquid injection or the Thermo Scientific™ TriPlus™ RSH autosampler for extra automated sample handling features. **[23]** as shown in figure below (5).

Figure 5: show the Thermo Scientific TSQ 8000 Evo Triple Quadrupole GC-MS/MS Easy operation.

The Thermo Scientific 8000 Evo Triple Quadrupole GC-MS/MS is the perfect choice. This saves time and lowers laboratory expenses. It is intended for standard uses, such as the detection of environmental and food pollutants and the quantification or confirmation of trace compounds in forensic toxicology and sports doping labs. Analysts can examine their target chemicals at lower concentration levels which boosts result confidence. The innovative device also saves money and time in the lab by decreasing errors and eliminating the need for repeated injections of the same sample. Additionally, the instrument's increased selectivity makes it possible to analyze more complicated matrices, which minimizes the requirement for sample preparation and increases throughput and turnaround times.

7.2 Solid Samples

After weighing 10 g of the sample (solid, sediment, or building material), 40 ml of extraction solution and anhydrous Na_2SO_4 were added to a glass jar. After adding the solvent mixture (hexane and acetone), the glass jar was sealed with a Teflon*seal and subjected to a 20-minute sonication. After placing an aliquot of the sample extract in a Kuaderma-Danish apparatus, the extraction process was repeated with an additional 40 milliliters of the extraction solvent mixture. The first extraction aliquot was supplemented with a second extraction aliquot. Three to four milliliters of the extract were evaporated, and the remaining amount was then evaporated under a mild nitrogen stream.

7.3 Method Setup

A technique was created for the TSQ 8000 Mass Spectrometer and the Thermo Scientific TRACE 1310 Gas Chromatograph (table 3) **[24].**

Table 3: Suggested conditions for instruments [25].

TRACE 1310 GC	
Injection Volume:	1µL
Liner:	Sittee baffed liner (P/N 4S3T2120)
Carrier Gas:	He, constant flow, 1,15ml/min
Column Type:	20 m, 18 mm ID, 0.18 µ df, T6-XLBMS (P/N 26079-5780)
Column Oven:	initial 60^0C, HOLD 1 min Pamp30.0^0C min to 200^0C Ramp 10.0^0C min to 320^0C Hold 2.0^0C
Transfer Liner:	320^0C

TRACE 1310 GC PTV program	
Injector Temperature:	80^0C, 0.1 min, 600^0C/min to transfer step
PTV Inject:	00^0C, 0,1 min. 600^0C/min to clean step
PTV Transfer:	320^0C, 5 min, 870^0C/min to clean step
PTV Quart:	325^0C, 15 min, clean flow 25 ml/min.

TSQ 8000 Mass Spectrometer in EL MODE	
Source Temperature:	350^0C
Ionization:	B, 70 eV
Emission Current:	50 µ/L
Resolution:	01 normal
Collection Gas:	Argon

7.4 Hydrocarbon Concentrations

The amounts of PAHs varied with soil depth, with surface samples showing higher quantities because of direct exposure. Severe pollution was indicated by concentrations more than 50 ng/ml, especially in the vicinity of petrol stations.

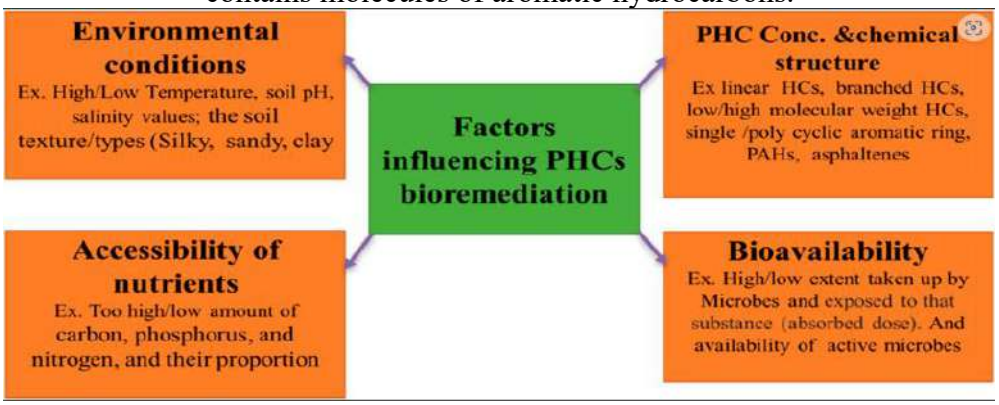
Table 4: illustrating the proper levels of multi-Loop aromatic hydrocarbons in the soil [26]

Policy	Essential hydrocarbons concentration	Description
Unfortunate	<1	Natural background levels
Speed pollution	10-1	The possibility of a light-minded effect
Medium pollution	50-10	The impact of the mold
Pollution	>50	Big dangerous

7.5 Soil Physicochemical Properties

Since of the weak microbial content in the sandy and clay soils, which is compatible with the ratio of C:N high and lower surface structure, the soil types have a significant impact on the success of its vital therapy. For example, the silk, sandy, and clay soils have a higher rate of hydrocarbon decomposition.

Figure 6: lists the primary types of parameters influencing the vital therapy of soil that contains molecules of aromatic hydrocarbons.



7.6Microbial Identification

- **Method of isolated Bacteria**

Selective medium (Chocolate Agar, MacConkey Agar, Blood Agar, and CLED Agar) were used to isolate the bacteria. Gramme staining and API 20E biochemical assays were used for identification. They were cultivated in nutritional media and the types of samples (silt, sand, black, and brown) after bacteria were isolated from nine samples contaminated with soil contaminated with petroleum and its derivatives from locations on Al-Zet Street (Iraq) and places where car oils are changed and lubricated using light oil and sterile dark plastic bags. In order to determine whether the soil is acidic or alkaline, a method that analyses the separated hydrogen ions by measuring the PH of a glass electrode—which varies from 6 to 4—is used.

Additionally, it detects alkaline soil, which may be addressed with gypsum, when the amount of negative hydroxyl ions in the soil solution is less than 7, indicates that the soil is in an absorption state, and results in a drop in fertility.

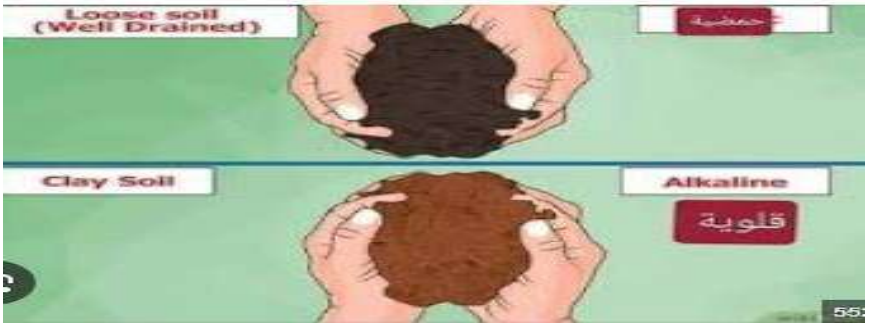


Figure 7: Shows the different between its acids and the alkaline of the soil.

Figure 8: explain the Strip tape to test its acids**.**

The samples were directly inspected for the disease-causing germs under a microscope. Microbial dyes, which are unique substances that give microorganisms like bacteria color and make them visible, were applied to the majority of the samples. If bacteria are present, they are recognizable by their size, shape, and color.

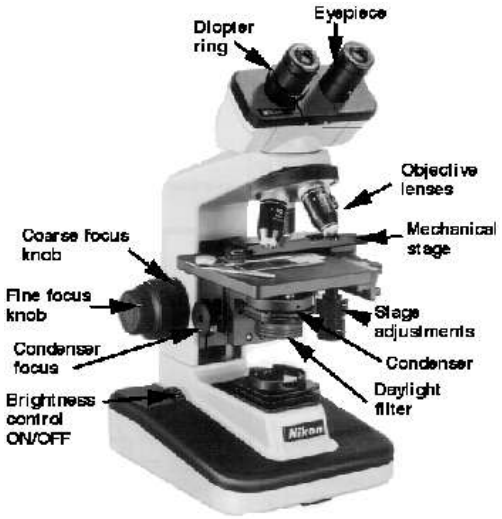


Figure 9: Show the type of microscope used for bacterial detection

➢ **Microbial Strain [27]**

Strain: According to **[28]** strain is an organic-colored substance that can form bonds with other materials to give it its color.

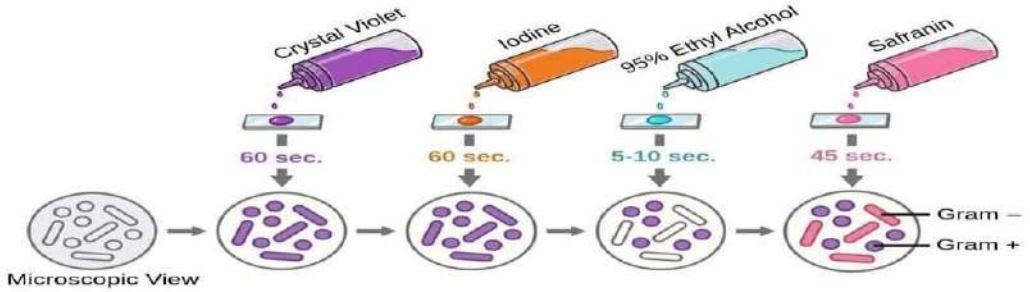


Figure 10: Shows a gram strain [29].

API 20E: It is a tool used in laboratory diagnosis to identify the types of intestinal bacteria and other types of Gram-negative bacteria.it depends on a set of mini biochemical tests that are investigated in 20 chamber's contained different reagents.

Figure 11: Shows the API 20E [30].

7.7 Compounds of hydrocarbons for biological degradation

When pollutants in the midst of tiny areas are reached, bioremediation takes place **[31].** However, the removal of the contaminants from the pollution site is significantly hampered when their bioremediation readiness is lower for one reason or another. Adsorption and active transport, which transfer chemical compounds to the microbial cell surface **[32]**, are two mechanisms that contribute to the hydrocarbons in the soil not being prepared for the areas under analysis **[33].** in their investigation of the soil's vital disintegration, which results in its notions in the minutes Not suitable for residential areas, this is thought to be resistant to essential dismantling. A few factors are thought to influence the chemical compounds in the soil, the most significant of which are the following groups:(1) Setting up the soil and determining its organic matter, humidity, and pH content (2) The connection between the chemicals and soil minutes (3) Soil humus-related vehicles, which are typically challenging to disassemble. from precise areas of dirt**. [34]; [35].**

7.8 Degradation of aromatic hydrocarbons by aerobic microbes

Pollutants found in all atmospheres are polycyclic aromatic hydrocarbons **[36].** It is widely distributed throughout the several ecosystems that support these chemicals' continuous presence in the environment **[37].** As a result of incomplete combustion of organic materials from human activities, it is considered to be one of the most dangerous environmental contaminants found in soil and its derivatives, which have the capacity to persist for a long time without degrading **[38].** Oxidation, bioaccumulation, and bacterial degradation are biological and abiotic processes that are closely linked to the fate of their existence in the environment **[39].** As seen in Figure (12), which is taken from Ref. **[40],** the biodegradation of aromatic chemicals is achieved by splitting the benzene ring via an intracellular enzymatic process. The aromatic ring is hydroxylated by the oxygenase enzyme to produce acisdihydrodiol, which is then converted to an intermediate by a dehydrogenase **[41].** The oxygenase enzymes break down the aromatic rings by using either meta-cleavage or orthocleavage to create new chemicals such catechol's, which subsequently change into certain citric acid cycle intermediates **[42].** Research has demonstrated that aromatic compounds with two or three rings can be broken down by bacteria, monooxygenase, and dioxygenase enzymes. This results in a cyclic fission that reduces the complexity of catechol's, increases their exposure, and facilitates their consumption **[43].**

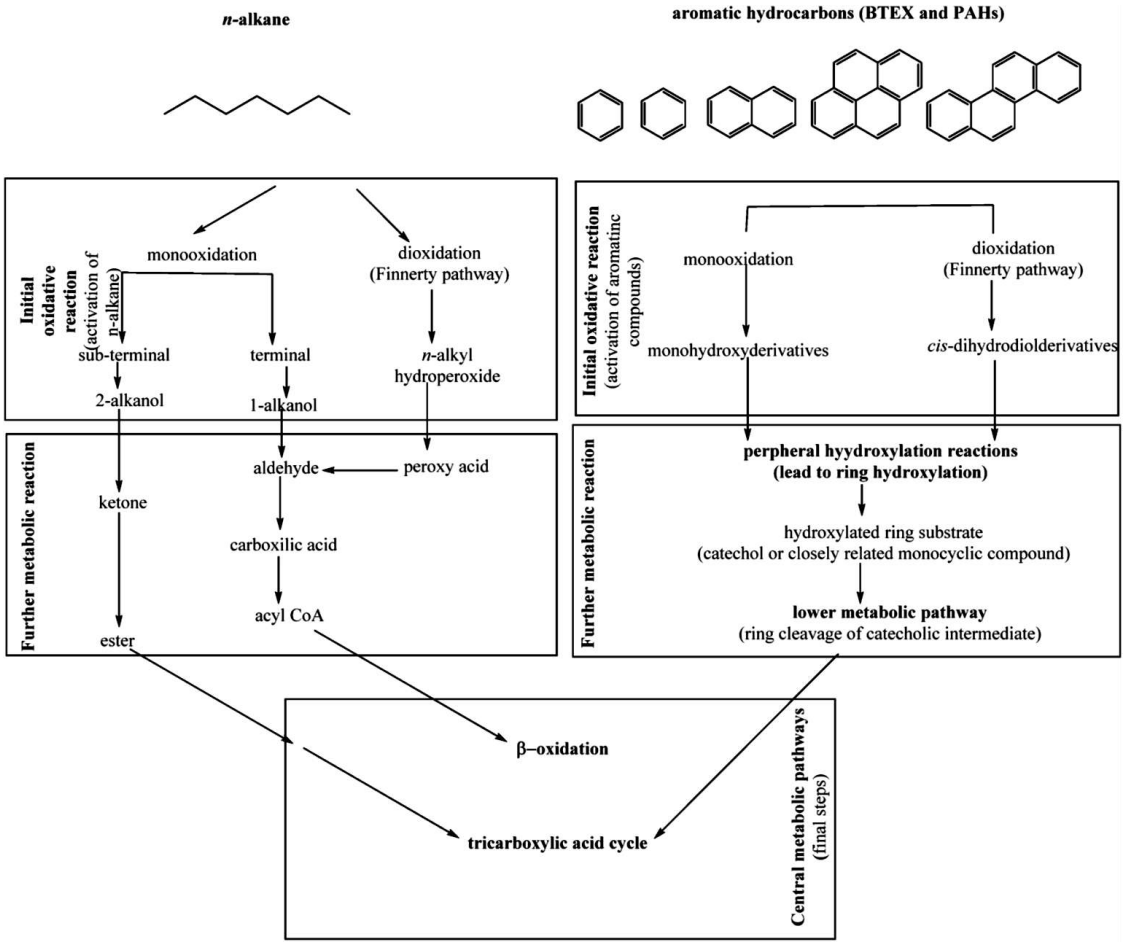


Figure. 12. Bacteria's aerobic routes for breaking down aliphatic and aromatic hydrocarbons were modified from Ref. **[39].**

7.9 Bacterial biodegradation is affected by several factors.

Microorganisms' competition for scarce carbon sources, exposure to predators and primitives, and the occurrence of antagonistic interactions among themselves all have an impact on the process of hydrocarbon biodegradation, as Table 5 illustrates in detail. Furthermore, the concentration of these pollutants and the quantity of catalyst present determine how quickly hydrocarbons contaminated with the soil decompose **[44].** Since it depends on calculating the rate of decomposition of the contaminated hydrocarbons in the soil via the number of organisms analyzed to note the occurrence of a low rate of decomposition of hydrocarbons in the event of a decrease in the number of bacteria in the soil, the microbial strains that predominate in the polluted soil are the ones that have the capacity to survive in the presence of these pollutants and use them as a source of metabolism and growth. In order to combat the lack of microorganisms, the opposite happens if there are enough active bacteria, which can be achieved by a dynamic increase that involves pollination the soil with appropriate strains of degrading pollutants **[45,46].** The following are some of the many parameters that significantly impact microbial bio-degradation:

Table 5: Factors influencing the biodegradation process of bacteria.

No.	Factors	Process of biodegradation
1	Nutrient	The microbial use of hydrocarbons to promote bacterial growth and activity requires nutrients **[47].** In addition to the other elements that bacteria require, such as nitrogen and phosphorus, carbon is one of the most crucial nutrients that must be supplied in order for hydrocarbons to be broken down efficiently. In addition to supporting the proper operation of all cell structure and metabolic processes, oil spills frequently result in nutrients becoming closed off and unavailable to bacteria, which are necessary for the production of the precursors for new microbial cells. **[48];** observed that excessive levels of nutrients like as nitrogen and phosphorous in the soil can have a detrimental effect on the biodegradability of hydrocarbons, which in turn inhibits the bacterial activity that breaks down hydrocarbons. According to [50]. the availability of inorganic nutrients, particularly phosphorus and nitrogen, is a crucial regulatory factor in soil hydrocarbon decomposition since it significantly increases the rates at which hydrocarbons biodegrade in the soil **[49–50].**
2	Oxygen Level	Depending on their needs, certain bacteria do not require oxygen, which speeds up the rate of biodegradation. Oxygen is a significant parameter for breathing the aerobic bacteria that it needs the most. According to Sihag et al. (2014), oxygen can generally improve the metabolism of hydrocarbons, however both anaerobic and aerobic conditions are needed for biodegradation to occur. The biodegradation process proceeds more quickly under aerobic conditions than under anaerobic ones **[51–52].**
3	Temperature	The temperature is a significant factor in the formation of hydrocarbons and determining the survival of bacteria **[53].** The increase in temperature increases the solubility of hydrocarbons, as well as reduces the viscosity of the oil, accelerates the spread of

		hydrophobic pollutants and enhances the rates of hydrocarbon decomposition **[54].** Conversely, if the temperature decreases, this leads to a delay in the biodegradation process **[55].** The optimum biodegradation temperature for oil is from 30 to 37 ◦C for isolated bacteria, and the maximum rate of biodegradation of oil in the soil have been obtained at a temperature ranging from 30 to 40 ◦C **[55–56]**
4	Ph- Value	Depending on the degree of alkalinity, acidity, or neutrality of the compounds, the pH is one of the key factors influencing the proliferation and activity of bacteria in the soil, where metabolism is impacted, as well as the breakdown and removal of pollutants **[57]**. Even if the pH values changed only little, both high and low pH values had an effect on the biodegradation process of petroleum pollutants **[58].** because certain enzymes that bacteria create to carry out the biodegradation process function at a particular pH level [166]. According to **[59]** the majority of bacterial species prefer alkaline pH above natural pH for growth **[60].**
5	Salinity	Salinity has a significant impact on many areas activity levels. The biodegradation process is significantly impacted by the proliferation and variety of microorganisms, which are influenced by salt **[62,61].** Reduced availability of organic compounds and a change in osmotic pressure, which results in decreased solubility and the occurrence of so-called salting, are caused by the high concentrations of salt creating a particular pressure that creates an unsuitable environment for different bacteria due to the nutrient shutdown **[64,63]**. The decline in microbial respiration was attributed by **[65]** to a decline in the vital treatment rate **[66–67].** Because it inhibits the activity of the main enzymes in the microbial system and causes osmotic shock events in certain bacteria, which inhibit the biological structure of large molecules, plasma dissolution, and many physiological processes, high concentrations of sodium chloride in the soil have a detrimental effect on the deterioration of crude oil **[68].**

7.10 Impact of oil pollution on living organisms

Leaks or spills of oil have an impact on the soil, seriously harming the ecology and living things **[68–69]**. According to **[70]** oil contamination of the soil results in sterility and subsequently alters its composition, microbiological characteristics, and physicochemical properties. This causes plants to grow more slowly because the soil loses its fertility and its capacity to absorb and hold onto water **[70,71]**. For instance, **[72]** reported that acute exposure to hydrocarbons causes a variety of diseases, such as dermatitis, arrhythmia, acidosis, and encephalopathy **[75].** These effects as a result of the oil spill led to a decline in agricultural productivity, which has detrimental effects on people's lives in terms of the economy **[73].** Regarding the specific carcinogenic effects of certain petroleum hydrocarbons, studies have shown that working people are more likely to develop lung, stomach, bladder, and liver cancers, as well as some neurological and reproductive effects **[75].** Three means of human exposure to these pollutants—skin contact, inhalation, and others—account for between 88 and 98 percent of pollution techniques, indicating that eating is the primary source of human exposure to these pollutants **[76].** Regarding the impact of aliphatic hydrocarbons, their presence in the soil

causes greasy patches that restrict the soil's ability to exchange nutrients and oxygen **[77].** Additionally, it might impact the human nervous system. resulting in weariness, headache, lightheadedness, transient paralysis of the limbs, limb numbness, and loss of consciousness **[74,78**]. Applying the best treatment to oiled locations will lower the dangers of pollutants, according to **[78].**

8.Result

8.1 Identified the Bacterial in Medical Laboratory

We started growing from the plates once they were produced (MacConkey Agar Blood Agar, CLED Agar), as we used two different cultivation methods.

1.Directed mothed

Figure 13: Shows the implant of samples from the soil directly.

2.In direct mothed: In this particular case, we made a suspension in the lab using N.S. Sodium acetate and placed it in a tube with soil on top of it in an equal-partition ratio of 1:1. We apply the soil suspension to four primary plants after it has been formed: Chocolate Agar, MacConkey Agar, Blood Agar, and CLED Agar.

Figure 14: Shows the preparation of the dilute at the rate of 1:1 in the indirectly method.

Following planting, as seen in the photos, we place the dishes in the incubator for a whole day. 24 hours later, we remove the dishes from the incubator to check for growth. We observed that there was no growth in the dishes following direct planting, but that there was growth following the completion of suspension. In order to get results, the indirect approach must be used.

Figure 15: Shows the types of bacteria created following the indirect method of implantation.

Following our indirect growth in the dishes, we use grain strain to identify or diagnose the bacteria that grew on them. To do this, we followed these steps:
After cleaning the slide, we apply a drop of regular silane, add colonies or 0.5 colonies, fix it over a flame, and proceed with the staining procedure.

Figure 16: Shows Gram Strain.

The process consists of four stages:
- ✓ We put the Crystal Foliate dye for a minute, then we wash it.
- ✓ We put the iodine dye for a minute; the we wash it.
- ✓ We put the alcohol for 60 seconds; the we wash it.
- ✓ We put the Safranin dye for a minute, then we wash it.

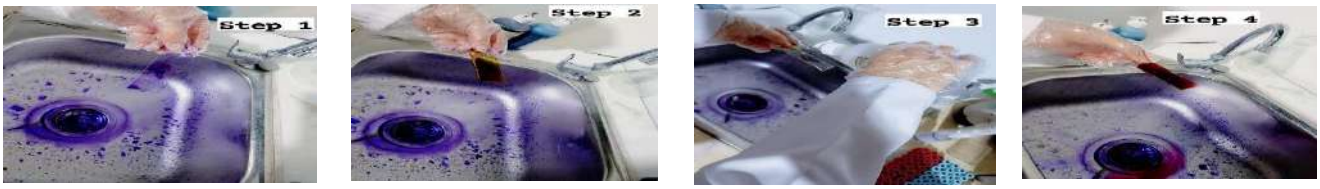


Figure 17: Explain the gum in Gram strain

And we examine it under the microscope by using the oil and read on its 100 lenes for the formats as shown in the following picture.
After staining and identifying the bacteria, we use a biochemical test to identify the type of bacteria that appeared to us.
Some important Bacterial general present

- ***Bacillus genus*:** represents 7–67% of the groups of bacteria and is one of the genera found in large quantities in soil that are easy to identify because they are organic, spore-forming, ***aerobiotic***, or experimental.

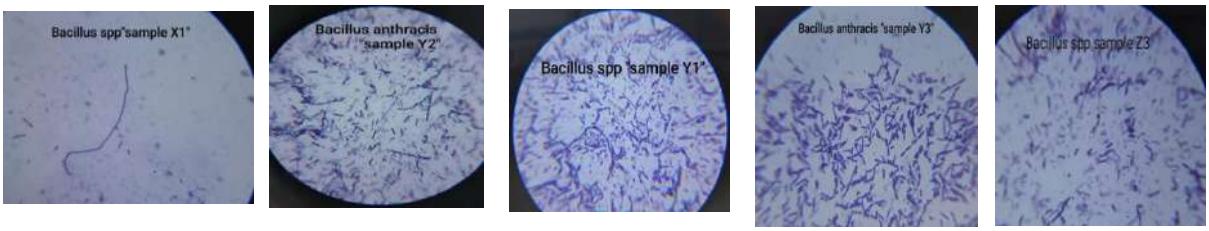


Figure 18: Bacillus genus

- ***Clostridium*:** The bacteria, which are of the gender C, are found in various soils with numbers ranging from 103 to 107 in grammes. They are massaged by estimating using the plants. and that the remining jars are developed in the suspended soil after it has been heated to 80 meters for 10 minutes, and then it is developed and multiplied in ***anaerobiotic conditions.***

Figure 19: Clostridium genus.

- ***Staphylococcus spp*:** The majority of soil contains anaerobic bacteria, some of which grow and make up less than 2% of the soil's bacterial population. Other ***anaerobic soil*** bacteria can grow with or without oxygen, and when the right conditions are met, they can produce nitrogen and other groups of anaerobic.

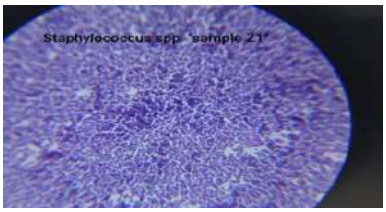


Figure 20: Staphylococcus spp genus.

- ***Pseudomonas:*** Two to twelve percent of the bacterial groups that convert nitrates into nitrogen lesions are antenna, and their functions include oxidation and organic vehicle analysis. It also secretes a lot of P. dentifrices to ***aerobic*** environments, including enzymes, the capacity to analyses sugars, organic and amino acids, and alcohol, as well as the ability to analyze pesticides.

Figure 21: Pseudomonas genus**.**

8.2 The PH: ranged between 8.00 to 9.96, which promoted the growth of microorganisms and the breakdown of hydrocarbons. Additionally, most microorganisms prefer neutral or alkaline conditions for the degradation of hydrocarbons **[79],** whereas the degree of acidity in the soil prevents the growth of ethical creatures **[80],** Thus, by increasing the PH, it is expected that the microbic population will be able to break down hydrocarbons.

Table 6: shows the hydrocarbons' PH values

Types Of Samples	**Surfaces Samples**			**Intermediate Samples**			**Deep Samples**		
Extracting Samples 5:1	Y1	X1	Z1	X2	Y2	Z2	X3	Y3	Z3
Values of PH	8.170	8.730	8.00	9.050	8.960	8.100	9.600	8.890	9.390

8.3 **Salinity:** Elevated TDS levels (73.3 to 1196 mg/L) decreased the efficiency of breakdown by inhibiting microbial enzymatic activity **[81].** Furthermore, because the high curative pressure of the shrouding environments inhibits the enzyme's release, raising the concentration of salt inhibits the growth of microorganisms **[82].** As a result, they are less able to absorb the abbachrocarbon molecule **[82].**

Table 7: shows the hydrocarbons' salinity values.

Extracting Samples 5:1	**Salinity TDS**	**Types Of Samples**
X1	663.000	**Surfaces**
Y1	1196.000	
Z1	263.000	
X2	331.000	Intermediate
Y2	118.000	
Z2	73.300	
X3	65.800	Deep
Y3	257.000	
Z3	105.300	

8.4 The measurement the concentration of hydrocarbons

Following the guidelines set forth in the practical section, a set of GC-MS analyses was prepared using the device's thermal program to analyze diluted solutions of standard polycyclic aromatic hydrocarbons (PAHs) with concentrations ranging from 10, 25, 50, 100, 200, 500, to 1000 µg/L. Each measurement was repeated three times to determine the retention times, and the response area was recorded for each concentration of the aromatic hydrocarbon mixture. Additionally, standard solutions of each polycyclic aromatic hydrocarbon were prepared separately, and calibration curves were drawn for each compound, as shown in the tables and calibration curve figures below.

Using these calibration curves, linear equations were computed to determine the concentrations of the compounds in soil samples collected randomly from surface, intermediate, and deep layers, allowing for the calculation of total PAH concentrations.

By comparing the mass of each aromatic compound with the mass of its corresponding ion in the mass spectrum, and utilizing the calibration curves, the concentrations of individual PAHs in each soil sample were accurately determined. As indicated in the tables, the results demonstrate the presence of these compounds at varying concentrations across different sites and collection times. It was observed that the concentrations of the target compounds were lower than those listed in the tables due to environmental factors during the measurement process, particularly the influence of salinity, pH, and hydrogen complexes.

1. Acenaphthylene

The results in the table and figure below demonstrate the measured response areas as (432, 1006, 226, 215, 886, 1114, 516, 41, 395). Additionally, it was observed that the intermediate layer (X2) did not produce a clear signal in the chromatography system when compared to other PAHs. The linear equation derived from the calibration curve, y=812627+205.031Xy = 812627 + 205.031Xy=812627+205.031X with R2=0.9994R^2 = 0.9994R2=0.9994, indicates a high degree of correlation, showing minimal deviation between the estimated and actual concentrations. The retention time for Acenaphthylene was determined to be 11.71 minutes.

Table (8) Shows the concentrations of the hydrocarbon compound (Acenaphthylene) compared to the standard focus of the different location of the soil near the oil station

Component Name: Acenapthylene curve index: Linear Weighing index: Equally Origin index: ignore Equation y= 812627+205.031*X R^2=0.9994										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	213065	1000.00	999.550	0%	0.0%		CAL 08	ng/ml	11.71
PAHS_100ng	Std Brached Sampl	32126	100.000	117.052	17%	0.0%		CAL 05	ng/ml	11.71

	e									
PAHS_10 ng	Std Brached Sample	3967	10.000	-20.287	303 -%	NA	Excluded	CAL 02	ng/ml	11.71
PAHS_200ng	Std Brached Sample	48170	200.000	195.304	-2%	0.0%		CAL 06	ng/ml	11.71
PAHS_25 ng	Std Brached Sample	10960	25.000	13.822	-45%	0.0%		CAL 03	ng/ml	11.71
PAHS_500ng	Std Brached Sample	192298	500.000	898.260	80%	NA	Excluded	CAL 07	ng/ml	11.71
PAHS_50 ng	Std Brached Sample	18229	50,000	49.272	-1%	0.0%		CAL 04	ng/ml	11.71
Y1 Surface	Unknown Sample	432	NA	37.528	NA	NA		NA	ng/m	11.71
X1 Surface	Unknown Sample	1006	NA	34.727	NA	NA		NA	ng/ml	11.71
Z1 Surface	Unknown Sample	226	NA	38.531	NA	NA		NA	ng/ml	11.71
Z3 deep	Unknown Sample	215	NA	38.587	NA	NA		NA	ng/ml	11.71
X3 deep	Unknown Sample	886	NA	35.311	NA	NA		NA	ng/ml	11.71

Z2 Intermediate	Unknown Sample	1114	NA	34.203	NA	NA		NA	ng/ml	11.72
Y2 Intermediate	Unknown Sample	516	NA	37.116	NA	NA		NA	ng/ml	11.71
Y3 deep	Unknown Sample	41	NA	39.432	NA	NA		NA	ng/ml	11.71
X2 Not clear	Unknown Sample	395	NA	37.709	NA	NA		NA	ng/ml	11.71

2. Fluoren

The results in the table (8) and figure (21) below demonstrated that are (625,1405,570,320,295,924,373,271,928). Additionally, the linear equation y=50785.7+1860.63*X R^2=0.9990 shows that there is no difference between the computed amount and the specific amount. Additionally, the retention period is 12.95, 12.96, and 12.97.

Table (9): Shows the concentrations of the hydrocarbon compound (Fluorene) compared to the standard focus of the different location of the soil near the oil stations.

Component Name: Flourene curve index: Linear Weighing index: Equally Origin index: ignore Equation y=50785.7+1860.63*X R^2=0.9990										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	141869	1000.000	103.542	-90%	0.0%		CAL 08	ng/ml	12.97
PAHS_100ng	Std Brached Sample	118770	100.000	91.128	-9%	0.0%		CAL 05	ng/ml	12.96
PAHS_10ng	Std Brached Sample	12338	10.000	33.926	239%	NA	Excluded	CAL 02	ng/ml	12.96

PAHS_200ng	Std Brached Sample	325952	200.000	202.478	1 %	0.0%		CAL 06	ng/ml	12.96
PAHS_25ng	Std Brached Sample	25940	25.000	41.236	65 %	0.0%		CAL 03	ng/ml	12.96
PAHS_500ng	Std Brached Sample	879827	500.000	500.160	0 %	NA	Excluded	CAL 07	ng/ml	12.95
PAHS_50ng	Std Brached Sample	53845	50,000	56.234	12 %	0.0%		CAL 04	ng/ml	12.96
Y1 Surface	Unknown Sample	652	NA	27.645	NA	NA		NA	ng/ml	12.96
X1 Surface	Unknown Sample	1405	NA	28.050	NA	NA		NA	ng/ml	12.95
Z1 Surface	Unknown Sample	570	NA	27.601	NA	NA		NA	ng/ml	12.97
Z3 deep	Unknown Sample	320	NA	27.467	NA	NA		NA	ng/ml	12.96
X3 deep	Unknown Sample	295	NA	27.453	NA	NA		NA	ng/ml	12.96
Z2 Intermediate	Unknown Sample	924	NA	27.792	NA	NA		NA	ng/ml	12.96
Y2 Intermediate	Unknown Sampl	373	NA	27.495	NA	NA		NA	ng/ml	12.96

	e									
Y3 deep	Unknown Sample	271	NA	327.441	NA	NA		NA	ng/ml	12.96
Not clear X2	Unknown Sample	928	NA	27.794	NA	NA		NA	ng/ml	12.96

3. Phenanthrene

The results in the table (9) and figure (21) below demonstrated that are (590,1047,563,497,589,641,666,489,395). Additionally, the linear equation y=737419+23323.8*X R^2=0.9989 indicates that there is no difference between the estimated amount and the specified amount. Additionally, the retention period is 15.36/15.37

Table (10): Shows the concentrations of the hydrocarbon compound (Phenanthrene) compared to the standard focus of the different location of the soil near the oil stations.

Component Name: Phenanthrene curve index: Linear Weighing index: Equally Origin index: ignore
Equation y=737419+23323.8*X R^2=0.9989

Firename	Sample Type	Area	Specific Amount	Calculated Amount	% Diff	%RSD-AMT	Peak Status	Level	Units	RT
PAHS_1000ng	Std Brached Sample	24076485	1000.000	1000.656	0 %	0.0%	Response High	CAL 08	ng/ml	15.35
PAHS_100ng	Std Brached Sample	3549429	100.000	120.564	21 %	0.0%		CAL 05	ng/ml	15.35
PAHS_10ng	Std Brached Sample	394887	10.000	-14.686	-247 %	NA	Excluded	CAL 02	ng/ml	15.36
PAHS_200ng	Std Brached Sample	5101322	200.000	187.101	-6 %	0.0%		CAL 06	ng/ml	15.35

PAHS_25 ng	Std Brached Sample	1056340	25.000	13.674	-45 %	0.0%		CAL 03	ng/ml	15.35
PAHS_500ng	Std Brached Sample	22914120	500.000	950.820	90 %	NA	Excluded	CAL 07	ng/ml	15.35
PAHS_50 ng	Std Brached Sample	1973689	50,000	53.005	6 %	0.0%		CAL 04	ng/ml	15.36
Y1 Surface	Unknown Sample	590	NA	31.591	NA	NA		NA	ng/ml	15.37
X1 Surface	Unknown Sample	1047	NA	31.572	NA	NA		NA	ng/ml	15.37
Z1 Surface	Unknown Sample	563	NA	31.593	NA	NA		NA	ng/ml	15.37
Z3 deep	Unknown Sample	497	NA	31.595	NA	NA		NA	ng/ml	15.37
X3 deep	Unknown Sample	589	NA	31.591	NA	NA		NA	ng/ml	15.37
Z2 Intermediate	Unknown Sample	641	NA	31.589	NA	NA		NA	ng/ml	15.37
Y2 Intermediate	Unknown Sample	666	NA	31.588	NA	NA		NA	ng/ml	15.36
Y3 deep	Unknown Sample	489	NA	31.596	NA	NA		NA	ng/ml	15.37

X2 Not clear	Unknown Sample	395	NA	31.600	NA	NA		NA	ng/ml	15.37

4. Antherathene

The results in the table (10) and figure (21) below demonstrated that are (380,453,445,393,372,961,881,1412,669). Additionally, the linear equation y=186788+20330*X R^2=0.9991 indicates that there is no difference between the estimated amount and the specified amount. Additionally, 15.43 is the retention time.

Table (11): Shows the concentrations of the hydrocarbon compound (Antherathene) compared to the standard focus of the different location of the soil near the oil stations.

Component Name Anthrathene: curve index: Linear Weighing index: Equally Origin index: ignore Equation y=186788+20330*X R^2=0.9991										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	20560905	1000.000	1002.320	0 %	0.0%	Response High	CAL 08	ng/ml	15.42
PAHS_100ng	Std Brached Sample	1962537	100.000	87.346	-13 %	0.0%		CAL 05	ng/ml	15.42
PAHS_10ng	Std Brached Sample	318136	10.000	6.461	-35 %	0.0%		CAL 02	ng/ml	15.42
PAHS_200ng	Std Brached Sample	4058785	200.000	190.456	-5 %	0.0%		CAL 06	ng/ml	15.42
PAHS_25ng	Std Brached Sample	803479	25.000	30.334	21 %	0.0%		CAL 03	ng/ml	15.42
PAHS_500ng	Std Brach	19479009	500.000	948.948	90 %	NA	Excluded	CAL 07	ng/ml	15.42

	ed Sample									
PAHS_50 ng	Std Brached Sample	1574100	50,000	68.239	36 %	0.0%		CAL 04	ng/ml	15.42
Y1 Surface	Unknown Sample	380	NA	9.169	NA	NA		NA	ng/ml	15.43
X1 Surface	Unknown Sample	453	NA	9.169	NA	NA		NA	ng/ml	15.43
Z1 Surface	Unknown Sample	445	NA	9.169	NA	NA		NA	ng/ml	15.43
Z3 deep	Unknown Sample	393	NA	9.169	NA	NA		NA	ng/ml	15.43
X3 deep	Unknown Sample	372	NA	9.169	NA	NA		NA	ng/ml	15.43
Z2 Intermediate	Unknown Sample	961	NA	9.169	NA	NA		NA	ng/ml	15.43
Y2 Intermediate	Unknown Sample	881	NA	9.169	NA	NA		NA	ng/ml	15.43
Y3 deep	Unknown Sample	1412	NA	9.169	NA	NA		NA	ng/ml	15.43
X2 Not clear	Unknown Sample	669	NA	9.169	NA	NA		NA	ng/ml	15.43

5. Pyrene

The results in the table (11) and figure (21) below demonstrated that are (66185,95647,21553,8258,29311,1116,7361,119550,8370). Additionally, the linear equation y=2145.02+6994.78*X R^2=0.9984 shows that there is no difference between the computed amount and the specified amount. Additionally, 19.95/19.96/19.94 is the retention time.

Table (12): Shows the concentrations of the hydrocarbon compound (Pyrene) compared to the standard focus of the different location of the soil near the oil stations.

Component Name: Pyrene curve index: Linear Weighing index: Equally Origin index: ignore Equation y=2145.02+6994.78*X R^2=0.9984										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Bracheed Sample	7013151	1000.000	1002.320	0 %	0.0%	Response High	CAL 08	ng/ml	19.35
PAHS_100ng	Std Bracheed Sample	1861229	100.000	122.818	23 %	0.0%		CAL 05	ng/ml	19.36
PAHS_10ng	Std Bracheed Sample	33624	10.000	4.500	-55 %	0.0%		CAL 02	ng/ml	19.36
PAHS_200ng	Std Bracheed Sample	1233280	200.000	176.008	-12 %	0.0%		CAL 06	ng/ml	19.36
PAHS_25ng	Std Bracheed Sample	167383	25.000	23.623	-6 %	0.0%		CAL 03	ng/ml	19.36
PAHS_500ng	Std Bracheed Sample	6503792	500.000	929.500	86 %	NA	Excluded	CAL 07	ng/ml	19.35
PAHS_50ng	Std Brache	391976	50,000	55.732	11 %	0.0%		CAL 04	ng/ml	19.35

	d Sample									
Y1 Surface	Unknown Sample	66185	NA	9.155	NA	NA		NA	ng/ml	19.95
X1 Surface	Unknown Sample	95647	NA	13.367	NA	NA		NA	ng/ml	19.96
Z1 Surface	Unknown Sample	21553	NA	2.775	NA	NA		NA	ng/ml	19.95
Z3 deep	Unknown Sample	8258	NA	0.874	NA	NA		NA	ng/ml	19.95
X3 deep	Unknown Sample	29311	NA	3.884	NA	NA		NA	ng/ml	19.95
Z2 Intermediate	Unknown Sample	11116	NA	1.282	NA	NA		NA	ng/ml	19.95
Y2 Intermediate	Unknown Sample	7361	NA	0.746	NA	NA		NA	ng/ml	19.85
Y3 deep	Unknown Sample	119550	NA	2.448	NA	NA		NA	ng/ml	19.95
X2 Not clear	Unknown Sample	8370	NA	0.890	NA	NA		NA	ng/ml	19.94

6. Benzoanthrat

The results in the table (12) and figure (21) below demonstrated that are (432,552,702,626,112,184,293,162,234). Additionally, the linear equation y=1.73394e+006+21900.3*X R^2=0.9944 indicates that there is no difference between the computed amount and the specific amount. Additionally, the retention period is 22.85.

Table (13): Shows the concentrations of the hydrocarbon compound (Benzoanthrat) compared to the standard focus of the different locations of the soil near the oil station

Component Name: Benzoanthrat curve index: Linear Weighing index: Equally Origin index: ignore Equation y=1.73394e+006+21900.3*X R^2=0.9944										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	20318911	1000.000	1006.965	1%	0.0%	Response High	CAL08	ng/ml	22.83
PAHS_100ng	Std Brached Sample	487785	100.000	101.447	1%	0.0%		CAL05	ng/ml	22.84
PAHS_10ng	Std Brached Sample	21250	10.000	80.145	701%	NA	Excluded	CAL02	ng/ml	22.85
PAHS_200ng	Std Brached Sample	1669537	200.000	155.408	-22%	0.0%		CAL06	ng/ml	23.08
PAHS_25ng	Std Brached Sample	69813	25.000	82.362	229%	NA	Excluded	CAL03	ng/ml	22.84
PAHS_500ng	Std Brached Sample	17763068	500.000	890.261	78%	NA	Excluded	CAL07	ng/ml	22.83
PAHS_50ng	Std Brached Sample	153437	50,000	86.180	72%	0.0%		CAL04	ng/ml	22.84
Y1 Surface	Unknown Sample	432	NA	79.194	NA	NA		NA	ng/ml	22.85
X1 Surface	Unknown Sample	552	NA	79.199	NA	NA		NA	ng/ml	22.85
Z1 Surface	Unknown Sample	702	NA	79.206	NA	NA		NA	ng/ml	22.85
Z3 deep	Unknown Sample	626	NA	79.203	NA	NA		NA	ng/ml	22.85
X3 deep	Unknown Sample	112	NA	79.179	NA	NA		NA	ng/ml	22.85
Z2 Intermediate	Unknown Sample	184	NA	79.183	NA	NA		NA	ng/ml	22.85
Y2 Intermediate	Unknown Sample	293	NA	79.188	NA	NA		NA	ng/ml	22.85
Y3 deep	Unknown Sample	162	NA	79.182	NA	NA		NA	ng/ml	22.85

X2 Not clear	Unknown Sample	234	NA	79.185	NA	NA		NA	ng/ml	22.85

7. Chyrsen

The results in the table (13) and figure (21) below demonstrated that, are 651,396,1011, 659,149,1939, 158, 569, and 204. Additionally, the linear equation y=-985238+21614.5*X R^2=0.9978 was observed. There is no difference between the computed amount and the specific amount. Additionally, the retention period is (23.09)

Table (14): Shows the concentrations of the hydrocarbon compound (Chyrsen) compared to the standard focus of the different location of the soil near the oil station

Component Name: Chyrsen Equation			**curve index: Linear Weighing index: Equally Origin index y=-985238+21614.5*X R^2=0.9978**							
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	206676259	1000.000	1002.174	0 %	0.0%	Response High	CAL08	ng/ml	23.07
PAHS_100ng	Std Brached Sample	543474	100.000	70.726	-29 %	0.0%		CAL05	ng/ml	23.07
PAHS_10ng	Std Brached Sample	29342	10.000	46.940	369 %	NA	Excluded	CAL02	ng/ml	23.08
PAHS_200ng	Std Brached Sample	854650	200.000	85.123	-57 %	NA	Excluded	CAL06	ng/ml	23.08
PAHS_25ng	Std Brached Sample	74899	25.000	49.047	96 %	0.0%		CAL03	ng/ml	23.08
PAHS_500ng	Std Brached Sample	18211767	500.000	888.153	78 %	NA	Excluded	CAL07	ng/ml	23.07
PAHS_50ng	Std Brached Sample	161474	50,000	53.053	6 %	0.0%		CAL04	ng/ml	23.08
Y1 Surface	Unknown Sample	651	NA	45.612	NA	NA		NA	ng/ml	23.09
X1 Surface	Unknown Sample	396	NA	45.601	NA	NA		NA	ng/ml	23.09
Z1 Surface	Unknown Sample	1011	NA	45.629	NA	NA		NA	ng/ml	23.09
Z3 deep	Unknown Sample	659	NA	45.613	NA	NA		NA	ng/ml	23.09
X3 deep	Unknown Sample	149	NA	45.589	NA	NA		NA	ng/ml	23.09

Z2 Intermediate	Unknown Sample	1939	NA	45.672	NA	NA		NA	ng/ml	23.08
Y2 Intermediate	Unknown Sample	158	NA	45.590	NA	NA		NA	ng/ml	23.09
Y3 deep	Unknown Sample	569	NA	45.609	NA	NA		NA	ng/ml	23.09
X2 Not clear	Unknown Sample	204	NA	44.592	NA	NA		NA	ng/ml	23.09

8. Benzo(b) FLOUR

The results in the table (14) and figure (21) below demonstrated that are also (296, 411, 370, 137, 327, 435, 308, 679, 117). Additionally, the linear equation y=-918230+15753*X R^2=0.9979 was observed. There is no difference between the computed amount and the specific amount. And the retention time is (26.05).

Table (15): Shows the concentrations of the hydrocarbon compound (Benzo(b) FLOUR) compared to the standard focus of the different location of the soil near the oil stations.

Component Name: Benzo(k)Flour curve index: Linear Weighing index: Equally Origin index: ignore Equation y=-918230+15753*X R^2=0.9979										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	14898835	1000.000	1004.051	0%	0.0%	Response High	CAL 08	ng/ml	26.03
PAHS_100ng	Std Brached Sample	562749	100.000	94.011	-6%	0.0%		CAL 05	ng/ml	26.04
PAHS_10ng	Std Brached Sample	29746	10.000	60.177	502%	NA	Excluded	CAL 02	ng/ml	26.05
PAHS_200ng	Std Brached Sample	1859719	200.000	176.341	-12%	0.0%		CAL 06	ng/ml	26.04

PAHS_25 ng	Std Brached Sample	135253	25.000	66.874	167 %	0.0%		CAL 03	ng/ml	26.04
PAHS_500ng	Std Brached Sample	11070854	500.000	761.055	52 %	NA	Excluded	CAL 07	ng/ml	26.04
PAHS_50 ng	Std Brached Sample	272667	50,000	75.597	51 %	0.0%		CAL 04	ng/ml	26.04
Y1 Surface	Unknown Sample	296	NA	58.307	NA	NA		NA	ng/ml	26.05
X1 Surface	Unknown Sample	411	NA	58.314	NA	NA		NA	ng/ml	26.05
Z1 Surface	Unknown Sample	370	NA	58.312	NA	NA		NA	ng/ml	26.05
Z3 deep	Unknown Sample	137	NA	58.297	NA	NA		NA	ng/ml	26.05
X3 deep	Unknown Sample	327	NA	58.309	NA	NA		NA	ng/ml	26.05
Z2 Intermediate	Unknown Sample	435	NA	58.316	NA	NA		NA	ng/ml	26.05
Y2 Intermediate	Unknown Sample	308	NA	58.308	NA	NA		NA	ng/ml	26.05
Y3 deep	Unknown Sample	679	NA	58.331	NA	NA		NA	ng/ml	26.05

X2 Not clear	Unknown Sample	117	NA	58.296	NA	NA		NA	ng/ml	26.05

9. Benzo(K)Flourane

The results in the table (15) and figure (21) below demonstrated that are also (268, 172, 49, 599, 576, 368, 216, 348, 64). Additionally, the linear equation y=-558169+124*X R^2=0.9971 shows that there is no difference between the computed amount and the specified amount.And the retention time is (26.13).

Table 16: Shows the concentrations of the hydrocarbon compound (Benzo(K)Flourane) compared to the standard focus of the different location of the soil near the oil station.

Component Name: Benzo(k)Flouran curve index: Linear Weighing index: Equally Origine index: ignore Equation y=-558169+124*X R^2=0.9971										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	13732051	1000.000	1006.235	1%	0.0%	Response High	CAL 08	ng/ml	26.11
PAHS_100ng	Std Brached Sample	667509	100.000	86.305	-14%	0.0%		CAL 05	ng/ml	26.12
PAHS_10ng	Std Brached Sample	28577	10.000	41.315	313%	NA	Excluded	CAL 02	ng/ml	26.13
PAHS_200ng	Std Brached Sample	1848680	200.000	169.477	-15%	0.0%		CAL 06	ng/ml	26.04
PAHS_25ng	Std Brached Sample	172002	25.000	51.415	106%	0.0%		CAL 03	ng/ml	26.12
PAHS_500ng	Std Brache	10523321	500.000	780.295	56%	NA	Excluded	CAL 07	ng/ml	26.12

	d Sample									
PAHS_50 ng	Std Brached Sample	316202	50,000	61.568	23 %	0.0%		CAL 04	ng/ml	26.12
Y1 Surface	Unknown Sample	268	NA	39.322	NA	NA		NA	ng/ml	26.13
X1 Surface	Unknown Sample	172	NA	39.315	NA	NA		NA	ng/ml	26.13
Z1 Surface	Unknown Sample	49	NA	39.307	NA	NA		NA	ng/ml	26.13
Z3 deep	Unknown Sample	599	NA	39.345	NA	NA		NA	ng/ml	26.13
X3 deep	Unknown Sample	576	NA	39.344	NA	NA		NA	ng/ml	26.14
Z2 Intermediate	Unknown Sample	368	NA	39.329	NA	NA		NA	ng/ml	26.13
Y2 Intermediate	Unknown Sample	216	NA	39.318	NA	NA		NA	ng/mL	26.13
Y3 deep	Unknown Sample	348	NA	39.328	NA	NA		NA	ng/mL	26.13
X2 Not clear	Unknown Sample	64	NA	39.308	NA	NA		NA	ng/mL	26.13

10. Benzopyrene

The results in the table (16) and figure (21) below demonstrated that are also (802, 229, 2288, 811, 1162, 1068, 686, 1667, 612). The concentrations of the samples (Surfaces, Intermediate, Deep) of (X1,Y1,Z1,X2,Y2,Y3,Y3,Z3) are also as follows. Additionally, the linear equation y=-382285+8716*X R^2=0.9952 was observed. There is no difference between the computed amount and the specific amount. And the retention time is (27.23).

The table (17): Shows the concentrations of the hydrocarbon compound (Benzopyrene) compared to the standard focus of the different location of the soil near the oil station

Component Name: Benzo(a)pyrene curve index : Linear Weighing index: Equally Origin index: ignore Equation y=-382285+8716*X R^2=0.9952										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Bracked Sample	8406919	1000.000	1008.334	1%	0.0%	Response High	CAL 08	ng/ml	27.20
PAHS_100ng	Std Bracked Sample	458903	100.000	96.505	-3%	0.0%		CAL 05	ng/ml	27.21
PAHS_10ng	Std Bracked Sample	31312	10.000	47.450	374%	NA	Excluded	CAL 02	ng/ml	27.22
PAHS_200ng	Std Bracked Sample	948725	200.000	152.699	-24%	0.0%		CAL 06	ng/ml	27.21
PAHS_25ng	Std Bracked Sample	61269	25.000	50.886	104%	0.0%		CAL 03	ng/ml	27.21
PAHS_500ng	Std Bracked Sample	6397403	500.000	777.794	56%	NA	Excluded	CAL 07	ng/ml	27.20
PAHS_50ng	Std Brach	198030	50,000	66.576	33%	0.0%		CAL 04	ng/ml	27.21

	ed Sample									
Y1 Surface	Unknown Sample	802	NA	43.949	NA	NA		NA	ng/ml	27.22
X1 Surface	Unknown Sample	229	NA	43.884	NA	NA		NA	ng/ml	27.23
Z1 Surface	Unknown Sample	2288	NA	44.120	NA	NA		NA	ng/ml	27.23
Z3 deep	Unknown Sample	811	NA	43.950	NA	NA		NA	ng/ml	27.23
X3 deep	Unknown Sample	1162	NA	43.991	NA	NA		NA	ng/ml	27.23
Z2 Intermediate	Unknown Sample	1068	NA	43.980	NA	NA		NA	ng/ml	27.23
Y2 Intermediate	Unknown Sample	686	NA	43.936	NA	NA		NA	ng/ml	27.2
Y3 deep	Unknown Sample	1667	NA	44.049	NA	NA		NA	ng/ml	27.2
X2 Not clear	Unknown Sample	612	NA	43.928	NA	NA		NA	ng/ml	27.2

11. pyrene 1,2,3-cd

The results in the table (17) and figure (21) below demonstrated that are 806, 978, 845, 1165, 1230, 1333, 704, 841, 985. Additionally, the linear equation y=-51709.3+1971.147*X R^2=0 was observed. There is no difference between the computed amount and the specific amount. Additionally, the retention time is (30,76/30.77).

Table (18): shows the concentrations of the hydrocarbon compound (pyrene 1,2,3-cd) compared to the standard focus of the different location of the soil near the oil station.

Component Name Indopyrene(1,2,3 cd) p **curve index: Linear** **Weighing index: Equally** **Origine index : ignore** **Equation** **y=-51709.3+1971.147*X R^2=0.9992**										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Branched Sample	191890 9	1000.000	999.566	0%	0.0%		CAL 08	ng/ml	30.78
PAHS_100ng	Std Branched Sample	168356	100.000	111.625	12 %	NA	Excluded	CAL 05	ng/ml	30.75
PAHS_10ng	Std Branched Sample	2413	10.000	27.453	175 %	NA	Excluded	CAL 02	ng/ml	30.77
PAHS_200ng	Std Branched Sample	28454	200.000	40.661	-80 %	0.0%		CAL 06	ng/ml	30.75
PAHS_25ng	Std Branched Sample	10931	25.000	31.774	27 %	0.0%		CAL 03	ng/ml	30.77
PAHS_500ng	Std Branched Sample	1473803	500.000	773.793	55 %	NA	Excluded	CAL 07	ng/ml	30.78
PAHS_50ng	Std Branched Sample	11447	50,000	32.035	-36 %	0.0%		CAL 04	ng/ml	30.78
Y1 Surface	Unknown Sampl	806	NA	26.638	NA	NA		NA	ng/ml	30.77

	e									
X1 Surface	Unknown Sample	978	NA	26.725	NA	NA		NA	ng/ml	30.76
Z1 Surface	Unknown Sample	845	NA	26.657	NA	NA		NA	ng/ml	30.77
Z3 deep	Unknown Sample	1165	NA	26.820	NA	NA		NA	ng/ml	30.77
X3 deep	Unknown Sample	1230	NA	26.853	NA	NA		NA	ng/ml	30.78
Z2 Intermediate	Unknown Sample	1333	NA	26.905	NA	NA		NA	ng/ml	30.77
Y2 Intermediate	Unknown Sample	704	NA	26.586	NA	NA		NA	ng/ml	30.77
Y3 deep	Unknown Sample	841	NA	26.655	NA	NA		NA	ng/ml	30.77
X2 Not clear	Unknown Sample	985	NA	26.729	NA	NA		NA	ng/ml	30.77

12. diBenzo(a,h)Anth

The results in the table (18) and figure (21) below demonstrated that are (1349, 637, 370, 1350, 1971, 912, 754, 507, 651). Additionally, the linear equation y=-55120+1975*X R^2=0.9993 indicates that there is no difference between the calculated amount and the specific amount. And the retention time is (30.79)

Table (19): Shows the concentrations of the hydrocarbon compound (diBenzo(a,h)Anth) compared to the standard focus of the different location of the soil near the oil stations.

Component Name: dIBenzo(a,h) Anth curve index: Linear Weighing index: Equally Origin index: ignore Equation y=-55120+1975*X R^2=0.9993										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	1918909	1000.000	999.407	0%	0.0%		CAL08	ng/ml	30.78
PAHS_100ng	Std Brached Sample	168356	100.000	113.141	13%	0.0%		CAL05	ng/ml	30.75
PAHS_10ng	Std Brached Sample	3688	10.000	29.773	198%	NA	Excluded	CAL02	ng/ml	30.79
PAHS_200ng	Std Brached Sample	208295	200.000	133.361	-33%	N%	Excluded	CAL06	ng/ml	30.79
PAHS_25ng	Std Brached Sample	1668	25.000	28.751	15%	0.0%		CAL03	ng/ml	30.79
PAHS_500ng	Std Brached Sample	1473803	500.000	774.060	55%	NA	Excluded	CAL07	ng/ml	30.78
PAHS_50ng	Std Brached Sample	11447	50,000	33.701	-33%	0.0%		CAL04	ng/ml	30.78
Y1 Surface	Unknown Sample	1349	NA	28.589	NA	NA		NA	ng/ml	30.79
X1 Surface	Unknown Sample	637	NA	28.228	NA	NA		NA	ng/ml	30.78
Z1 Surface	Unknown Sample	370	NA	28.093	NA	NA		NA	ng/ml	30.79
Z3 deep	Unknown Sample	1350	NA	28.589	NA	NA		NA	ng/ml	30.79
X3 deep	Unknown Sample	1971	NA	28.904	NA	NA		NA	ng/ml	30.79
Z2 Intermediate	Unknown Sample	912	NA	28.368	NA	NA		NA	ng/ml	30.79
Y2 Intermediat	Unknown Sample	754	NA	28.288	NA	NA		NA	ng/ml	30.79
Y3 deep	Unknown Sample	507	NA	28.163	NA	NA		NA	ng/ml	30.79
X2 Not clear	Unknown Sample	651	NA	28.236	NA	NA		NA	ng/ml	30.79

13. Benzo[ghi]perylene

The results in the table (19) and figure (21) below demonstrated that are also as follows. Additionally, the linear equation y=-382285+8716*X R^2=0.9952 was observed. There is no

difference between the computed amount and the specific amount. And the retention time is (27,22/27.23/27.24).

Table (20): Shows the concentrations of the hydrocarbon compound (Benzo[ghi]perylene) compared to the standard focus of the different location of the soil near the oil stations.

Component Name: Benzo(ghiperylene curve index: Linear Weighing index: Equally Origin index: ignore Equation y=-382285+8716*X R^2=0.9952										
Firename	**Sample Type**	**Area**	**Specific Amount**	**Calculated Amount**	**% Diff**	**%RSD-AMT**	**Peak Status**	**Level**	**Units**	**RT**
PAHS_1000ng	Std Brached Sample	8406919	1000.000	1008.334	1%	0.0%	Response High	CAL 08	ng/ml	27.20
PAHS_100ng	Std Brached Sample	458903	100.000	96.505	-3%	0.0%		CAL 05	ng/ml	27.21
PAHS_10ng	Std Brached Sample	31312	10.000	47.450	374%	NA	Excluded	CAL 02	ng/ml	27.22
PAHS_200ng	Std Brached Sample	948725	200.000	152.699	-24%	0.0%		CAL 06	ng/ml	27.21
PAHS_25ng	Std Brached Sample	61269	25.000	50.886	104%	0.0%		CAL 03	ng/ml	27.21
PAHS_500ng	Std Brached Sample	6397403	500.000	777.794	56%	NA	Excluded	CAL 07	ng/ml	27.20
PAHS_50ng	Std Brached Sample	198030	50,000	66.576	33%	0.0%		CAL 04	ng/ml	27.21

	e									
Y1 Surface	Unknown Sample	802	NA	43.949	NA	NA		NA	ng/ml	27.22
X1 Surface	Unknown Sample	229	NA	43.884	NA	NA		NA	ng/ml	27.23
Z1 Surface	Unknown Sample	2288	NA	44.120	NA	NA		NA	ng/ml	27.23
Z3 deep	Unknown Sample	811	NA	43.950	NA	NA		NA	ng/ml	27.23
X3 deep	Unknown Sample	1162	NA	43.991	NA	NA		NA	ng/ml	27.23
Z2 Intermediate	Unknown Sample	1068	NA	43.980	NA	NA		NA	ng/ml	27.23
Y2 Intermediate	Unknown Sample	686	NA	43.936	NA	NA		NA	ng/ml	27.23
Y3 deep	Unknown Sample	1667	NA	44.049	NA	NA		NA	ng/ml	27.24
X2 Not clear	Unknown Sample	612	NA	43.928	NA	NA		NA	ng/ml	27.23

To calculate the concentrations of hydrocarbons using linear equation, the linear bodies are used and the relationship can be expressed between concentration C And responding to the analysis (Such as the area under the curved in chromatography by the following equation The linear slope is typically used to calculate the concentration of hydrocarbons using a linear equation.

Linear equation [83]
Y= mx + b

Y = Response (such as the area under the curved and absorption)

X= Focus (hydrocarbons concentration usually with unit's mg/l or mg/Kg

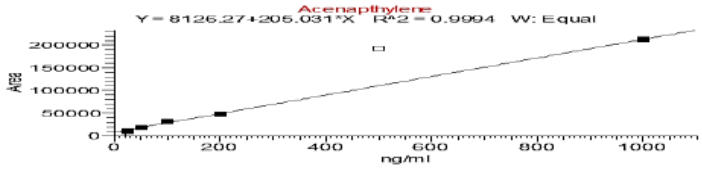
m = The inclination that represents the change in the represents the change in the response to each unit of focus

b = The parts of tabs represent the response when the focus is zero

If the concentrations of aromatic hydrocarbons in the soil do not reach the standard concentrations or cannot be mitigated, you can follow the following steps **(1)** Use more sensitive techniques such as spectral analysis (GC-MS) or high-performance liquid chromatography(HPLC) as high accuracy in measuring low concentrations **(2)** also re-analyzing samples using discreet analytical methods **(3)** You can return a new set of standards that include less concentrations, which allows the determination of the concentrations more accurate **(4)** The standard levels, so make a comprehensive evaluation of the risks to determine what if these levels represent an environment **(5)** analysis of physical and chemical properties of samples, which helps in understanding how these characteristics affect the concentrations of aromatic acedobens in the plural, These studies can include the analysis of the ability to exchange ions and the degree of acid and distribute the size of the grades. Environment such as the use of vegetable coal to improve the quality **(6)** also verify environmental factors such as the temperature, pressure and sample type that may be erected in the results **(7)** comparison of the concentrations to determine the differences **(8)** biological analysis and some bacterium species analysis and some the aromatic acidarbons,where you use it as a source of energy and lunch material which helps in cleaning polluted soils **(9)** The bacteria can be used effectively in the processes of processing polluted soil with aromatic hydrocarbons such as biotechnology technology and also the stains of factors such as salinity and PH on bacteria activity and its ability to degrades PAHs.
The TSQ 8000 Evo Triple Quadrupole GC-MS/MS system from Thermo Scientific is used to identify petroleum hydrocarbons in soil samples, particularly those with concentrations between C12 and C22.Given the anticipated lower amounts of petroleum hydrocarbons in the samples, a calibration curve is created for low values, ranging from 200 ng/L to 1000 ng/L. For accurate quantification, this curve is essential
interpretation of the Graph: The calibration curve for acenaphthylene is depicted in the graph, with the x-axis showing the concentration (ng/ml) and the y-axis showing the area under the chromatogram's curve

The formula

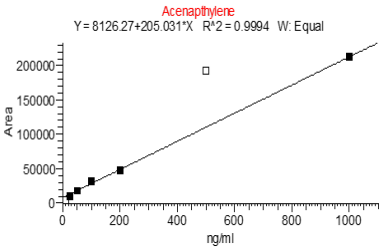
Y = 8126.27 + 205.031×X



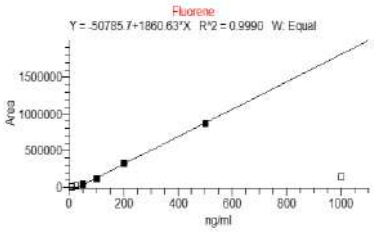
shows a good linear correlation with an R2 value of 0.9994, indicating high measurement accuracy and dependability. The plotted points (black squares) indicate measured concentrations, confirming the method's ability to identify low soil hydrocarbon levels.

This approach is essential for soil quality evaluation and environmental monitoring, particularly in regions where petroleum products may have an influence. Accurate hydrocarbon quantification in soil samples is made possible by the defined calibration curve, which provides crucial information for environmental managemen

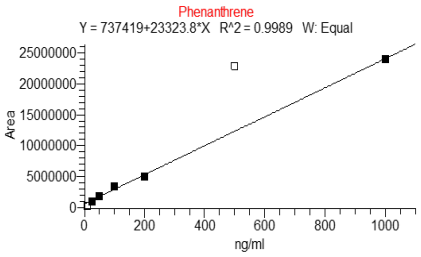
Thermo Scientific TSQ 8000 Evo Triple Quadrupole GC-MS/MS was used to determine the presence of petroleum hydrocarbons in soils. Simple operation (Soil quality: Gas chromatography for determining the hydrocarbon concentration in the C12–C22 range) [84]. The methodology outlined in the standard has a measurement range of 10 ng DM to 1000ng DM. A calibration curve was created for low concentrations between 200 mg/L and 1000 mg/L since the examined chemicals in the soil samples had lower anticipated values. The calibrated calibration curve is displayed in the graph below (Fig.22).



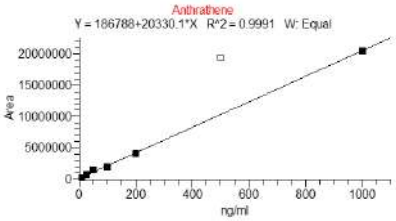
1-Acenapthylene



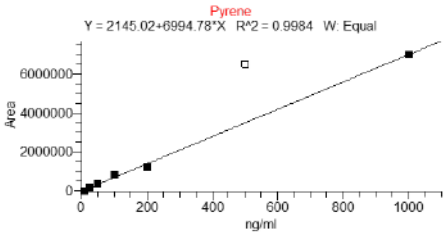
2-Fluorene



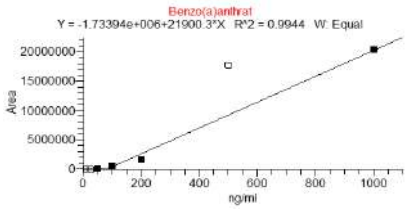
3-Phenanthrene



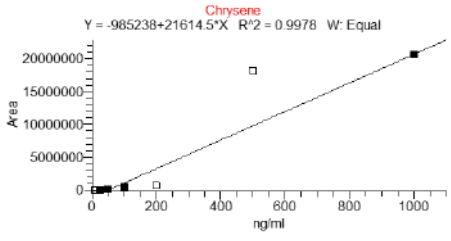
4-Anthrathene



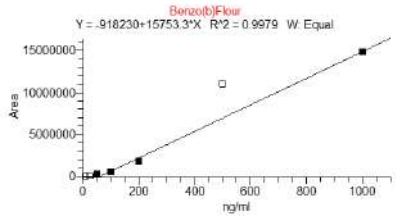
5-Pyrene



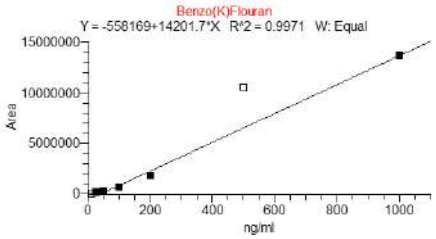
6-Benzo(a)anthrat



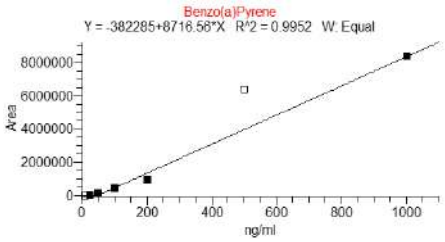
7- Chrysene



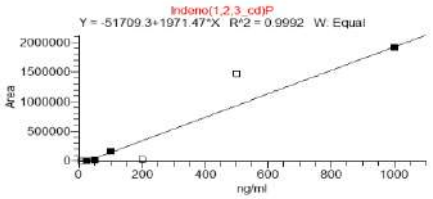
8-Benzo(b)Flour



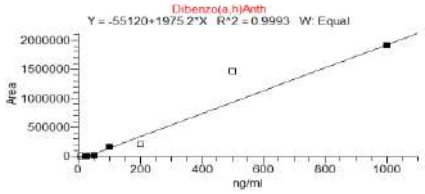
9- Benzo(k)Flouran



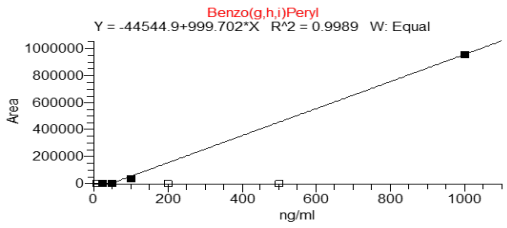
10-Benzo(a)Pyrene



11-Indeno(1,2,3_cd)p



12-Dibenzo(a,h)Anth



13-Benzo(g,h,i)Penyl

Figure (22): shows A mineral oil mixture standard's calibration curve with n-heptane as the hydrocarbon solvent.

Chromatographic analysis was performed using a gas chromatograph equipped with a column type with a 20 m, 18 mm ID, 0.18 df, T6-XLBMS (P/N 26079-5780), Carrier Gas: He, constant flow, 1,15 ml/min, and Collection Gas: argon. A single sample's analysis took 36 minutes, and it followed the following temperature program: To transfer step, 80^0C, 0.1 min, 6000C/min, 0^0C, and 1 min. 600^0C/min to clean the step, 320^0C for 5 minutes, 870^0C/min to clean the step,

and 325^{0}C for 15 minutes to clean the flow 25 milliliters per minute, following confirmation of the area under the curve findings from the three replications, values with a difference more than 502% were rejected. From the rest, the average was determined. The final result was computed using the formula provided in the standard **[84]**, accounting for the mass of the sample under analysis, its dry matter content, and the data from the calibration curve.

9. Bioremediation Potential

Under anaerobic conditions, the detected bacterial strains demonstrated notable biodegradation capabilities, indicating their possible use in environmental cleanup. Numerous flaws in physical and chemical action, such as cost, procedural complexity, organizational burden, and lack of total deterioration, have led to a recent surge in interest in the treatment of multiple episodes using biological methods, which are thought to be environmentally friendly.

The interlocutor-contaminated pursuit consists of both on-site (land transplantation, biomed motivation, vital quantity, fertilization, and vegetarian therapy) and off-site (vital reactions) pitons.

10. Conclusion

The study provides a valuable analysis of hydrocarbons pollution in Benghazi, while highlighting the role of bacteria in vital treatment, with this, you need improvements in systematic tempering and visual condition, in addition to expanding the scope of samples to generalize the results. The recommendations made are a process towards pollution management, but its benefit requires constant institutional and research support

The primary aim of this study is to provide current information on the various hydrocarbons present in the environment, exposure routes, and the damage they do to ecosystems. Hydrocarbon sources and their impacts on the environment and human health have been thoroughly examined and presented. The mutagenic, teratogenic, and carcinogenic qualities of hydrocarbons have sparked serious concerns in light of this research, which can originate from natural or man-made sources. Microbes that live in the ecosystem degrade petroleum hydrocarbons and other related pollutants in a complicated process. In addition to the amount of petroleum hydrocarbons present, the quantitative and qualitative changes of these pollutants are dependent on their origin, as well as seasonal and ambient environmental changes. This includes the PH and Salinity and change in properties of Benghazi' soil. And the concentrations, toxicity, and sources of essential aromatic compounds that are a major threat to human health and the ecosystem are assessed in this study. The Libyan environmental agency developed 13 aromatic compounds as vehicles for human diseases, particularly benzo(a) pyrene and the first class in terms of the damage caused by it. Performance and a minute represented in Thermo were used to analyze the data, and some methods have been proposed to decompose the multi-episodes hydrocarbons in order to treat the polluted soil that is currently being studied visually.

I. Reducing petroleum hydrocarbons at the source through resource conservation, effective oil use, and processing
II. Evaluation of the cytotoxicity and environmental effects of petroleum-based hydrocarbons, with an emphasis on the long term.
III. The weathering factors play an important factor on the bioremediation of these hydrocarbons; hence the impact of climate change on the characteristics of these pollutants and their elimination process should be addressed.

IV. Improving of the efficiency and increasing the quantity of the bacterial species involved in the bioremediation process should be studied via genetic area. Since weathering variables are crucial to the bioremediation of these hydrocarbons, it is vital to address how climate change is affecting the properties of these pollutants and the process of getting rid of them.

V. Genetic engineering should be investigated to increase the number of bacterial species involved in the bioremediation process and improve its effectiveness.

11. Recommendations

- Implement bioremediation strategies using indigenous bacteria.
- Monitor hydrocarbon levels in urban soils periodically.
- Promote sustainable waste management to prevent petroleum pollution.
- Conduct further research on microbial enzymatic pathways for enhanced biodegradation.
- The location and depth of the sample collection determine the present amount of contamination in the soil environment.
- An unchecked rise in soil contamination is caused by improper ground protection and inadequate area development, such as unpaved parking spaces.
- Property owners should be forced to adapt their properties to the new laws as a result of the changes in the way the area is developed.
- To stop the deterioration of the soil environment, it should be continuously monitored.

12. Negative

1. Sampling samples

The collection of samples was limited to limited locations on the street. Which may not reflect the complete geographical distribution of pollution in Benghazi.

2. Lack of Clarity of some systematic details

The absence of accurate details about the methods of insulation of bacteria (such as the used media) and the API 20E tests, which is difficult to repeat the experience

3. Problems of Visual Coordination

Some tables contained (Such as domain 1(on errors in alignment or text format)

4.Weak references challenge

Some references such as ([1] and [2]) are not related to the subject of the study. Also, the absence of modern references after (2020) weakens the credibility of the results.

13.Recommendations for future improvement

1. Expanding the scope of the study

Collecting samples from a variety of sites in Benghazi (such as industrial and residential areas) for the analysis of the coexistence in pollution.

2. Documenting methodology accurately

Clarify the steps to isolate bacteria (such as transmission and custody conditions) and explain biochemical tests (such as API 20E) in detail.

3.**Improve visual condition**

Using program such as GraphPad to create clear and organized newspapers and charts with numbered shapes and tables.

4.**Challenged Ai-Marah**

Including modern references (within 5 years) focusing on techniques of biological analysis of hydrocarbons and vital processing applications in polluted environments.

5.**Performing applied field studies**
Test of the isolated bacterial breeds in real circumstances (such as the contaminated soil fields) to measure their practical performance.
6.**Health risk analysis**
Add the department that evaluates the health risks caused by exposure to hydrocarbons, (such as calculating refineries to document the human impact and the environment).

14. Reference

[1] Sankar, T. K., Kumar, A., Mahto, D. K., Das, K. C., Narayan, P., Fukate, M., ... & Ambade, B. (2023). The health risk and source assessment of polycyclic aromatic hydrocarbons (PAHs) in the soil of industrial cities in India. *Toxics*, *11*(6), 515.
[2] Zhang, X. Y., Wu, H. J., Yu, B. S., Rosales-Guzmán, C., Zhu, Z. H., Hu, X. P., ... & Zhu, S. N. (2023). Real-time superresolution interferometric measurement enabled by structured nonlinear optics. *Laser & Photonics Reviews*, *17*(6), 2200967.
[3] Yang, Z., Chen, Z., Xin, Q., & Lee, K. (2024). Assessment of risk for aromatic hydrocarbons resulting from subsea Blowouts: A case study in eastern Canada. *Environment International*, *194*, 109136.
[4] Chopade, P., Chopade, N., Zhao, Z., Mitragotri, S., Liao, R., & Chandran Suja, V. (2023). Alzheimer's and Parkinson's disease therapies in the clinic. *Bioengineering & Translational Medicine*, *8*(1), e10367.
[5] Nagkirti, P. D., Engineer, A. S., & Dhakephalkar, P. K. (2021). Xylanimonas oleitrophica sp. nov., a novel petroleum hydrocarbon degrading bacterium isolated from an Indian oil reservoir. *Antonie Van Leeuwenhoek*, *114*, 129-136.
[6] Gupta, G., Chandra, A., Varjani, S. J., Banerjee, C., & Kumar, V. (2018). Role of biosurfactants in enhancing the microbial degradation of pyrene. *Bioremediation: Applications for environmental protection and management*, 375-386.
[7] Guo, Z. D., Wang, Z. Y., Zhang, S. F., Li, X., Li, L., Li, C., ... & Chen, W. (2020). Aerosol and surface distribution of severe acute respiratory syndrome coronavirus 2 in hospital wards, Wuhan, China, 2020. *Emerging infectious diseases*, *26*(7), 1586.
[8] Chaudhary, P. K., & Kim, S. (2021). An insight into GPCR and G-proteins as cancer drivers. *Cells*, *10*(12), 3288.
[9] Mrozik, A., Piotrowska-Seget, Z., & Labuzek, S. (2003). Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. *Polish Journal of Environmental Studies*, *12*(1).
[10] Abdel-Shafy, H. I., & Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egyptian journal of petroleum*, *25*(1), 107-123.
[11] Pandey, D., & Pandey, V. C. (2016). Sacred plants from ancient to modern era: Traditional worshipping towards plants conservation. *Tropical Plant Research*, *3*(1), 136-141.
[12] Sims, A. W., Sabadell, G. P., Lam, C. W., Segal, D. C., Bireta, P., & Thomas, D. (2023). Self-sustaining smouldering combustion for the treatment of industrial oily waste. *Waste Management & Research*, *41*(3), 713-722.
[13] Mrozik, A., & Piotrowska-Seget, Z. (2010). Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. *Microbiological research*, *165*(5), 363-375.
[14] Oualha, R., Barhoumi, M., Marzouki, S., Harigua-Souiai, E., Ben Ahmed, M., & Guizani, I. (2019). Infection of human neutrophils with Leishmania infantum or Leishmania major

strains triggers activation and differential cytokines release. *Frontiers in cellular and infection microbiology*, *9*, 153.
[15] Chebbi, A., Hentati, D., Zaghden, H., Baccar, N., Rezgui, F., Chalbi, M., ... & Chamkha, M. (2017). Polycyclic aromatic hydrocarbon degradation and biosurfactant production by a newly isolated Pseudomonas sp. strain from used motor oil-contaminated soil. *International Biodeterioration & Biodegradation*, *122*, 128-140.
[16] Medić, S., Anastassopoulou, C., Lozanov-Crvenković, Z., Dragnić, N., Petrović, V., Ristić, M., ... & Ioannidis, J. P. (2023). Incidence, risk, and severity of SARS-CoV-2 reinfections in children and adolescents between March 2020 and July 2022 in Serbia. *JAMA Network Open*, *6*(2), e2255779-e2255779.
[17] Nzila, A. (2018). Current status of the degradation of aliphatic and aromatic petroleum hydrocarbons by thermophilic microbes and future perspectives. *International journal of environmental research and public health*, *15*(12), 2782.
[18] Lebov, J., Grieger, K., Womack, D., Zaccaro, D., Whitehead, N., Kowalcyk, B., & MacDonald, P. D. (2017). A framework for One Health research. *One Health*, *3*, 44-50.
[19] Zamberlan, A., Gioachin, F., & Barbieri, P. (2024). Hiring intentions at the intersection of gender, parenthood, and social status. A factorial survey experiment in the UK labour market. *European Sociological Review*, jcae043.
[20] Hatzinger, P. B., & Lippincott, D. R. (2019). Field demonstration of N-Nitrosodimethylamine (NDMA) treatment in groundwater using propane biosparging. *Water Research*, *164*, 114923.
[21] Almouallem, W., Michel, J., Dorge, S., Joyeux, C., Trouvé, G., & Le Nouen, D. (2023). A comparative study of the sorption of O-PAHs and PAHs onto soils to understand their transport in soils and groundwater. *Journal of Environmental Sciences*, *124*, 61-75.
[22] Bai, X., Sun, H., Sun, J., & Zhu, Z. (2022). Efficient removal of sixteen priority polycyclic aromatic hydrocarbons from textile dyeing sludge using electrochemical Fe2+-activated peroxymonosulfate oxidation-A green pretreatment strategy for textile dyeing sludge toxicity reduction. *Journal of Hazardous Materials*, *435*, 129087.
[23] Borman, P. T., Uijtewaal, P., Snyder, J., Allen, B., Atienza, C. K., Woodhead, P., ... & Fast, M. F. Demonstration of motion-compensated volumetric modulated arc radiotherapy on an MR-linac. *Physics and Imaging in Radiation Oncology*.
[24] Ma, L., Liu, M., Wang, N., Wang, L., Yang, Y., & Wang, H. (2020). Room-level fall detection based on ultra-wideband (UWB) monostatic radar and convolutional long short-term memory (LSTM). *Sensors*, *20*(4), 1105.
[25] Hadeen, X., Rowe, C., Brand, M., Comley, R., Das, S., Wurster, C. M., ... & Bird, M. I. (2025). Comparing pollen and n-alkane carbon isotope records in a tropical lacustrine environment. *Quaternary Science Reviews*, *351*, 109204.
[26] Soukarieh, B., El Hawari, K., El Husseini, M., Budzinski, H., & Jaber, F. (2018). Impact of Lebanese practices in industry, agriculture and urbanization on soil toxicity. Evaluation of the Polycyclic Aromatic Hydrocarbons (PAHs) levels in soil. *Chemosphere*, *210*, 85-92.
[27] Dilawer Issa, K., & Dlshad Muhsin, M. (2024). Beta–Lactam Drug Resistance Pattern in Staphylococcus aureus Isolates: A Review. *EURASIAN JOURNAL OF SCIENCE AND ENGINEERING*.
[28] Quintanilla-Villanueva, G. E., Luna-Moreno, D., Núñez-Salas, R. E., Rodríguez-Delgado, M. M., & Villarreal-Chiu, J. F. (2024). Inactivation of Aspergillus Species and Degradation of Aflatoxins in Water Using Photocatalysis and Titanium Dioxide. *Processes*, *12*(12), 2673.

[29] AwladWadair, A. L. I., Ahmed, M. S., Mahran, E., Mohammed, A., Alkarousi, M., Altoubi, K., ... & Sultan, S. (2024). Emerging and re-emerging Zoonotic Viral Diseases Affecting Farm Animals in the Sultanate of Oman: A review of Current Situation. *SVU-International Journal of Veterinary Sciences*, *7*(4), 18-33.
[30] Woods, T. R., White, J., & Koutlas, I. (2023). Fungal lesions of the oral mucosa diagnosis and management. *Oral and Maxillofacial Surgery Clinics*, *35*(2), 271-281.
[31] Guerin, W. F., & Boyd, S. A. (1992). Differential bioavailability of soil-sorbed naphthalene to two bacterial species. *Applied and Environmental Microbiology*, *58*(4), 1142-1152.
[32] Alexander, M. (1991). ES&T Research Needs. *Environmental science & technology*, *25*(12), 1972-1973.
[33] Yang, L., Lai, C. T., & Shieh, W. K. (2000). Biodegradation of dispersed diesel fuel under high salinity conditions. *Water Research*, *34*(13), 3303-3314.
[34] Manilal, V. B., & Alexander, M. (1991). Factors affecting the microbial degradation of phenanthrene in soil. *Applied Microbiology and Biotechnology*, *35*, 401-405.
[35] Mahmoud, G. A. E., & Bagy, M. M. K. (2018). Microbial degradation of petroleum hydrocarbons. *Microbial action on hydrocarbons*, 299-320.
[36] Truskewycz, A., Gundry, T. D., Khudur, L. S., Kolobaric, A., Taha, M., Aburto-Medina, A., ... & Shahsavari, E. (2019). Petroleum hydrocarbon contamination in terrestrial ecosystems—fate and microbial responses. *Molecules*, *24*(18), 3400.
[37] Imron, M. F., & Titah, H. S. (2018). Optimization of diesel biodegradation by Vibrio alginolyticus using Box-Behnken design. *Environmental Engineering Research*, *23*(4), 374-382.
[38] Moreno, R., & Rojo, F. (2017). Enzymes for aerobic degradation of alkanes in bacteria. *Aerobic utilization of hydrocarbons, oils, and lipids. Handbook of hydrocarbon and lipid microbiology/Ed. F. Rojo. Springer International Publishing*, 1-25.
[39] Sierra-Garcia, I. N., & de Oliveira, V. M. (2013). Microbial hydrocarbon degradation: efforts to understand biodegradation in petroleum reservoirs. *Biodegradation-engineering and technology*, *10*, 55920.
[40] Atalia, K. R., Buha, D. M., Bhavsar, K. A., & Shah, N. K. (2015). A review on composting of municipal solid waste. *Journal of Environmental Science, Toxicology and Food Technology*, *9*(5), 20-29.
[41] Ladygina, N., Dedyukhina, E. G., & Vainshtein, M. B. (2006). A review on microbial synthesis of hydrocarbons. *Process Biochemistry*, *41*(5), 1001-1014.
[42] Brown, D. M., Bonte, M., Gill, R., Dawick, J., & Boogaard, P. J. (2017). Heavy hydrocarbon fate and transport in the environment. *Quarterly Journal of Engineering Geology and Hydrogeology*, *50*(3), 333-346.
[43] Thapa, B., Kc, A. K., & Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Kathmandu university journal of science, engineering and technology*, *8*(1), 164-170.
[44] Abdelzaher, M. A., Hamouda, A. S., El-Kattan, I. M., & Baher, A. (2022). Laboratory study for accelerating the CKD mineral carbonation. *Egyptian Journal of Chemistry*, *65*(3), 491-499.
[45] Singh, C., & Lin, J. (2010). Bioaugmentation efficiency of diesel degradation by Bacillus pumilus JLB and Acinetobacter calcoaceticus LT1 in contaminated soils. *African Journal of Biotechnology*, *9*(41), 6881-6888.

[46] Singh, C., & Lin, J. (2010). Bioaugmentation efficiency of diesel degradation by Bacillus pumilus JLB and Acinetobacter calcoaceticus LT1 in contaminated soils. *African Journal of Biotechnology*, *9*(41), 6881-6888.
[47] Hameed, S. N., & Al-Farttoosy, A. H. (2022). Biodegradation of Carbendazim using four bacterial Strains. *Misan Journal for Academic Studies*, *21*(44), 346-358.
[48] Koshlaf, E., & Ball, A. S. (2017). Soil bioremediation approaches for petroleum hydrocarbon polluted environments. *AIMS microbiology*, *3*(1), 25.
[49] Brown, B., Mackintosh, G. H., Mann, A. J., Ritchie, P. E., & Tanner, R. J. (2007). 2017. *The Records of the Parliaments of Scotland to*, *1707*.
[50] Al-Tharwani, W. S., Mohammadali, M. T., & Abd al-Ridha, I. M. (2023). Evaluation of the efficiency of Trichoderma harzianum and Azotobacter chroococcum in biodegradation of glyphosate and measurement of its residual in soil by using HPLC. *Misan Journal of Academic Studies*, *22*(47).
[51] Macaulay, B. M. (2015). Understanding the behaviour of oil-degrading micro-organisms to enhance the microbial remediation of spilled petroleum. *Appl Ecol Environ Res*, *13*(1), 247-262.
[52] Wang, L. C., Lin, L. F., & Lai, S. O. (2009). Emissions of polycyclic aromatic hydrocarbons from fluidized and fixed bed incinerators disposing petrochemical industrial biological sludge. *Journal of hazardous materials*, *168*(1), 438-444.
[53] Wang, X., Feng, J., & Zhao, J. (2010). Effects of crude oil residuals on soil chemical properties in oil sites, Momoge Wetland, China. *Environmental monitoring and assessment*, *161*, 271-280.
[54] Abdelzaher, M. A. (2021). Experiential investigation on the effect of heavy fuel oil substitution by high sulfur petcoke on the physico-mechanical features and microstructure of white cement composites. *Engineering Research Express*, *3*(1), 015028.
[55] Wang, Q., Zhang, S., Li, Y., & Klassen, W. (2011). Potential approaches to improving biodegradation of hydrocarbons for bioremediation of crude oil pollution. *Journal of environmental protection*, *2*(01), 47.
[56] Zekri, A. Y., & Chaalal, O. (2005). Effect of temperature on biodegradation of crude oil. *Energy Sources*, *27*(1-2), 233-244.
[57] Si-Zhong, Y. A. N. G., Hui-Jun, J. I. N., Zhi, W. E. I., Rui-Xia, H. E., Yan-Jun, J. I., Xiu-Mei, L. I., & Shao-Peng, Y. U. (2009). Bioremediation of oil spills in cold environments: a review. *Pedosphere*, *19*(3), 371-381.
[58] Aleer, S., Adetutu, E. M., Makadia, T. H., Patil, S., & Ball, A. S. (2011). Harnessing the hydrocarbon-degrading potential of contaminated soils for the bioremediation of waste engine oil. *Water, Air, & Soil Pollution*, *218*, 121-130.
[59] Al-Hawash, A. B., Dragh, M. A., Li, S., Alhujaily, A., Abbood, H. A., Zhang, X., & Ma, F. (2018). Principles of microbial degradation of petroleum hydrocarbons in the environment. *Egyptian Journal of Aquatic Research*, *44*(2), 71-76.
[60] Ibrahim, H. M. (2016). Biodegradation of used engine oil by novel strains of Ochrobactrum anthropi HM-1 and Citrobacter freundii HM-2 isolated from oil-contaminated soil. *3 Biotech*, *6*(2), 226.
[61] Asira, E. E. (2013). Factors that determine bioremediation of organic compounds in the soil. *Acad J Interdiscip Stud*, *2*(13), 125.

[62] Hasan, A. O., Abu-Jrai, A., Turner, D., Tsolakis, A., Xu, H. M., Golunski, S. E., & Herreros, J. M. (2016). Control of harmful hydrocarbon species in the exhaust of modern advanced GDI engines. *Atmospheric Environment*, *129*, 210-217.
[63] Kurniawan, T. A., Yanyan, L., Ouyang, T., Albadarin, A. B., & Walker, G. (2018). BaTiO3/TiO2 composite-assisted photocatalytic degradation for removal of acetaminophen from synthetic wastewater under UV–vis irradiation. *Materials Science in Semiconductor Processing*, *73*, 42-50.
[64] Gao, W., Zhang, Y., Ramanujan, D., Ramani, K., Chen, Y., Williams, C. B., ... & Zavattieri, P. D. (2015). The status, challenges, and future of additive manufacturing in engineering. *Computer-aided design*, *69*, 65-89.
[65] Hussein, Z. S., Hamido, N., Hegazy, A. K., El-Dessouky, M. A., Mohamed, N. H., & Safwat, G. (2022). Phytoremediation of crude petroleum oil pollution: a review. *Egyptian Journal of Botany*, *62*(3), 611-640.
[66] Fathepure, B. Z. (2014). Recent studies in microbial degradation of petroleum hydrocarbons in hypersaline environments. *Frontiers in microbiology*, *5*, 173.
[67] Gao, Y. C., Wang, J. N., Guo, S. H., Hu, Y. L., Li, T. T., Mao, R., & Zeng, D. H. (2015). Effects of salinization and crude oil contamination on soil bacterial community structure in the Yellow River Delta region, China. *Applied Soil Ecology*, *86*, 165-173.
[68] Erdogan, E. E., Sahin, F., & Karaca, A. (2012). Determination of petroleum-degrading bacteria isolated from crude oil-contaminated soil in Turkey. *African Journal of Biotechnology*, *11*(21), 4853-4859.
[69] Al-Thakafy, N. T., Abdelzaher, M. A., Abdelzaher, H. G., Saleh, M. Y., Al-Enizzi, M. S., Saied, S. M., ... & Moghanm, F. S. (2024). A novel chalcone compound as a reagent for the validation of pharmaceutical cefotaxime sodium preparations. *Results in Chemistry*, *7*, 101387.
[70] Ikuesan, F. A. (2017). Microbial response to varying concentrations of crude oil pollution of agricultural soils in Ondo State, Nigeria. *Microb Res J Int*, *22*, 1-8.
[71] Ikuesan, F. A. (2017). Evaluation of crude oil biodegradation potentials of some indigenous soil microorganisms. *Journal of Scientific Research and Reports*, *13*(5), 1-9.
[72] Benjeddou, O., Ravindran, G., & Abdelzaher, M. A. (2023). Thermal and acoustic features of lightweight concrete based on marble wastes and expanded perlite aggregate. *Buildings*, *13*(4), 992.
[73] Tormoehlen, L. M., Tekulve, K. J., & Nañagas, K. A. (2014). Hydrocarbon toxicity: A review. *Clinical toxicology*, *52*(5), 479-489.
[74] Chorom, M., Sharifi, H. S., & Motamedi, H. (2010). Bioremediation of a crude oil-polluted soil by application of fertilizers.
[75] Kassenova, Z., Kozhabekov, S., Zhubanov, A., Galymzhan, A., & Seilkhanov, T. (2023). Synthesis of comb-like copolymers based on alkyl fumarates and their application as pour-point depressants for akshabulak crude oil. *Results in Engineering*, *17*, 100820.
[76] Chandra, S., Sharma, R., Singh, K., & Sharma, A. (2013). Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. *Annals of microbiology*, *63*, 417-431.

[77] Luch, A. (Ed.). (2005). *The carcinogenic effects of polycyclic aromatic hydrocarbons*. World Scientific.

[78] Muangchinda, C., Srisuwankarn, P., Boubpha, S., Chavanich, S., & Pinyakong, O. (2020). The effect of bioaugmentation with Exiguobacterium sp. AO-11 on crude oil removal and the bacterial community in sediment microcosms, and the development of a liquid ready-to-use inoculum. *Chemosphere*, *250*, 126303.

[79] Kumar, S. U., Kumar, D. T., Christopher, B. P., & Doss, C. G. P. (2020). The rise and impact of COVID-19 in India. *Frontiers in medicine*, *7*, 250.

[80] Ren, X., Wen, W., Fan, X., Hou, W., Su, B., Cai, P., ... & Zhang, Z. (2021). COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. *Cell*, *184*(7), 1895-1913.

[81] Zhu, X., Jackson, R. D., DeLucia, E. H., Tiedje, J. M., & Liang, C. (2020). The soil microbial carbon pump: From conceptual insights to empirical assessments. *Global Change Biology*, *26*(11), 6032-6039.

[82] Muangchinda, C., Srisuwankarn, P., Boubpha, S., Chavanich, S., & Pinyakong, O. (2020). The effect of bioaugmentation with Exiguobacterium sp. AO-11 on crude oil removal and the bacterial community in sediment microcosms, and the development of a liquid ready-to-use inoculum. *Chemosphere*, *250*, 126303.

[83] Galer-Tatarowicz, K., Littwin, M., Pazikowska-Sapota, G., Dembska, G., & Radke, B. (2017). Optymalizacja metody oznaczania węglowodorów ropopochodnych techniką chromatografii gazowej z detekcją płomieniowo-jonizacyjną (GC-FID) w środowiskowych próbkach stałych (gleba, osad denny, osad ściekowy, odpady). *Aparatura Badawcza i Dydaktyczna*, *22*, 277-284.

[84] Callén, M. S., Iturmendi, A., & López, J. M. (2014). Source apportionment of atmospheric PM2. 5-bound polycyclic aromatic hydrocarbons by a PMF receptor model. Assessment of potential risk for human health. *Environmental pollution*, *195*, 167-177.