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BIOREMEDIATION OF PAHS FROM INDUSTRIAL REFINERY EFFLUENTS TO HIGHLY VALUABLE TRANSFORMED COMPOUNDS

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in the environment, and most highly molecular weight PAHs cause mutagenic, teratogenic and potentially carcinogenic effects. The study aims to screen the bioremediation effects on PAHs to produce new compounds with industrial importance by-products contaminated water using liquid- liquid extraction (LLE) coupled with gas chromatography-mass spectroscopy (GC-MS). Two bacterial isolates out of 13 were selected from PAHs samples contaminated water collected from Kuantan River. PAHs were extracted using dichloromethane from refinery wastewater samples collected from BASF (Petronas chemicals Co.). GC-MS analysis revealed that, the PAHs of petroleum refinery were successfully biodegraded using the most potent bacterial isolates within 15 days incubation periods into new compounds (Guanidine derivative, etc.) with industrial applications compared to control. This research improves our understanding of

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processes contributing to PAHs degradation in petroleum refinery wastewater to new applications.

***Keywords**:* Petroleum effluents, GC-MS, Bioremediation, Biotransformation.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemicals. They represent an important concern due to their widespread distribution in the environment, their resistance to biodegradation, their potential to bioaccumulate and their harmful effects. deterioration of soil and water quality (Kadri et al., 2016). Potential treatments have been implemented to prevent further economic consequences and deterioration of soil and water quality. Among such treatments, bioremediation initiatives promise to deliver long lasting and low cost solutions for PAHs degradation. Biodegradation of hydrocarbons was carried out either by bacteria (Hamamura et al., 2013;Ferreira et al., 2015), fungi (Li et al., 2005;Young et al., 2015). Microorganisms such as bacteria, fungi, algae are reported for their ability to degrade hydrocarbon pollutants (Varjani & Upasani, 2013; Wilkes et al., 2016). Bacteria are reported as primary degraders and most active agents in petroleum pollutant degradation (Abbasian et al., 2015; Meckenstock et al., 2016). Bioremediation is an innovative technique, in which microorganisms mitigate, degrade or reduce hazardous organic pollutants to innocuous compounds such as CO_2, CH_4, H_2O and biomass without adversely affecting environment (Ron and Rosenberg, 2014). Biodegradation is one of the primary mechanisms for bioremediation in which oleophilic microbes are used for elimination of hydrocarbon pollutants from environment (Varjani et al., 2013; Macaulay & Rees, 2014; Varjani & Upasani, 2016). Petroleum refinery effluents (PRE) are wastes originating from industries primarily engaged in refining crude oil and manufacturing fuels, lubricants and petrochemical intermediates(Harry 1995).Basically, PRE are complexmatrices of organic pollutants, and it iswell established that photocatalytic degradation can completely mineralise oily and hydrocarbon-rich waste waters. Furthermore, all of the different types of organic substrate typically found in PRE are also mineralised; therefore, by default, PRE can be effectively treated(Diya'uddeen, et al., 2011).

Some microorganisms have ability to degrade aliphatics, some can degrade monoaromatics or polyaromatics while others degrade resins. Petroleum hydrocarbon pollutants degrading microorganisms. Bacterial sp. of genera Achromobacter, Acinetobacter, Azoarcus, Brevibacterium, Cellulomonas, Corynebacterium, Flavobacterium, Marinobacter, Micrococcus, Nocardia, Ochrobactrum, Pseudomonas, Stenotrophomaonas and Vibrio are reported as hydrocarbon degraders(Varjani et al., 2015; Varjani & Upasani, 2016).

PAH degradation depends on the environmental conditions, number and type of the microorganisms, nature and chemical structure of the chemical compound being degraded.
They are biodegraded/biotransformed into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic) and rate of biodegradation depends on pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium. A number of bacterial species are known to degrade PAHs and most of them are isolated from contaminated soil or sediments. Pseudomonas aeruginosa, Pseudomons fluoresens, Mycobacterium spp., Haemophilus spp.,

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Rhodococcus spp., Paenibacillus spp. are some of the are some of the commonly studied PAH-degrading bacteria. (Haritash & Kaushik 2009).

Chromatographic techniques, such as GC and HPLC, have been used to analyse PAHs in water samples (Garcıa et al., 2004;Mao et al., 2012) Due to complexity of the refinery wastewater, the water samples are not directly analysed using gas chromatography, gas chromatography-mass spectrometry, or high-performance liquid chromatography. Therefore, appropriate sample preconcentration procedures are required in order to obtain accurate data with good precision.(Song et al., 2016). The organic chemical composition was characterized using a gas chromatograph-mass spectrometer (GC–MS). The water sample pretreatment method and GC–MS detection condition are described in the supplementary material (Wang, Wang et al. 2016).

The present study aims to isolate and screening of bacterial isolates from Kuantan River contaminated water for bioremediation and transformation purposes to produce new compounds with highly medical and industrial importance.

MATERIALS AND METHODS

Sample Collection and Preparation

Refinery wastewater samples were collected from BASF (Petronas chemicals Co.) Kuantan, Pahang, 26100, Malaysia. Water samples (2L) were collected in glass bottles covered with screw caps and immediately sent to the laboratory for analysis and Liquid-Liquid extraction as described in APHA (Andrew etal.,1998) using Filters Fioroni paper (90 mm diameter) under Vacuum pump and then stored at 4 $^{\circ}$C .

Media Preparation

An enrichment medium was prepared as follows (g/ L): $MgSO_4$, 0. 2; $CaCl_2$, 0.02; NH_4NO_3 ,1.0; KH_2PO_4 ,1.0 ;K_2HPO_4, $FeCl_3$, 0.05 (Prabhakaran, Sureshbabu et al. 2014)into demineralized water. Nutrient agar was prepared for culturing of bacterial isolates by adding 23.0 g of nutrient agar to 1000 mL of demineralized water. The media was purchased from Hardy Diagnostics, USA. The nutrient broth media were prepared by adding 20.0 g of nutrient broth to 1000 mL of demineralized water. The media was purchased from Merck, Germany. All the media were autoclaved for 15 minutes at 121 ^{o}C.

Biodegradation Experiments

Bacterial suspension (25mL) was prepared using Nutrient Broth (NB) media in 50 mL falcon tube centrifuge and incubated at 37°C for 24h. The bacterial suspension was enriched in an enrichment medium as previously describe at pH 7.2 using PH meter (METTLER TOLEDO) and 0.75 mL of PAHs added into 25 mL, 5mLbacteria suspension and the incubated at 37^{o}C for 5, 10, 15 days. At the end of incubation period then APHs was extracted using dichloromethane and APHs sample (1:8 v/v) after that the sample were evaporated up to at least 3mL using rotary evaporator then absorbance of the APHs was recorded using a UV-VIS spectrometer (GENESYS 10S) at 245nm.

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Sample Extraction

The water sample 200 mL were extracted three time with 25 mL of dichloromethane in separating funnel (1:8v/v) was added and shaken vigorously for 2 min stay 30 min before two phase separation. The organic-phase was carefully poured into beaker and add 10 g Anhydrous sodium sulphate (annealed at 400 ^{o}C for 4h at heating rate 3 ^{o}C /min). The extract was concentrated to the volume of 2 mL using rotary evaporator speed 3, 40° C heating then analysed with Gas chromatograph.

Gas Chromatograph-mass Spectrometer (GC-MS) Analysis

Sample analysis was performed on (GC of Agilent technology 7890A with a 7693 auto sampler) equipped with a DB-5MS capillary column (30 m × 250 µm film thickness × 0.25 mm, Agilent), operating with helium carrier gas, coupled to an Agilent MSD 5975C mass spectrometer (MS). GC injector was operated in splitless mode. 1 µL aliquot was injected using an auto sampler. GC oven temperature of injector and detector is maintained at 250 °C and 325 °C respectively. Column is set at initial temperature of 70°C, this is held for 2 min, then be increased at 5°C/min to 280°C and held for 10 min . GC–MS was used for PAHs separation and identification Chromatographic conditions were chosen according to (Shao et al., 2015) with modify.

RESULT AND DISCUSSION

Isolation of PAHs-Degrading Bacteria

Two bacterial isolates out of 13 bacterial isolate were selected from (KR) the isolated strains were identified based biochemical tests the isolate first type Mycobacterium confluents (D) and bacteria (F) did not have ID. Then absorbance of the APHs was recorded using spectrophotometer at 245nm bacteria D X% degradation 27.27% with concentration of 166.15 µL/mL. While Bacteria F X% degradation 6.06 % concentration 214.61µL/mL Compared with control 0.297 A±0.001 concentration 228.46µL/mL (Table 1). Isolation of two new strains, Mycobacterium austroafricanum GTI-23 and M. vanbaalinii, has been reported by (Bogan et al., 2003; Moody et al., 2004), respectively. These strains have been shown to mineralize PAHs like fluorene and benzo[a]pyrene in soil as well as liquid mediums.

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Table 1: Screening of PAHs bioremediation concentration

No	Sample code	X%	Concentration µL/mL
1	Blank solvent	0.0	0.0
2	control	0.0	228.46
3	KRA_1	0.0	440.77
4	KRB_1	0.0	332.30
5	KRC_1	0.0	460.00
6	KRA_2	0.0	275.38
7	KRB_2	0.0	341.53
8	KRE_2	0.0	268.46
9	KRD_2	27.27	166.15
10	KRF_2	0.0	300.00
11	KRB_3	0.0	403.07
12	KRC_3	0.0	427.69
13	KRF_3	6.06	214.16
14	KRA_4	22.22	177.69
15	KRB_4	0.0	236.92

GC-MS analysis of dichloromethane extracts of refinery wastewater samples(RW). Showed the presence of 13 compounds, 8 of them belong to PAHs (Table 2 and Fig. 1). Samples showed the initial Ph at 11.55 and 7.9 for RW and Kuantan River (KR)respectively.

Growth and Biodegradation

Mycobacterium confluentis, bacteria F and DF were tested to grow on enrichment medium (EM) at different incubation periods 5, 10 and 15 days with 0.75 mL PAHs. The course of biodegradation of PAHs in the *Mycobacterium confluentis* enrichment medium (EM) table 1 shows that Screening the by-products produced using GC-MS Analysis from PAHs Bioremediation at different incubation periods. Bacteria *Mycobacterium confluentis* after 5days all the compound were degraded except trans-2,3-Methylenedioxy-b-methyl- b-nitrostyrene at Retention Time (RT) 43.965 and (PA) 3.45% respectively and bacteria F except Methyl 2-methyl-2-(methoxy-3-hydroxypropoxy)amino-propanoate at (RT) 40.275 and (PA) 4.93% respectively. While ,bacteria DF 8 out of 13 compounds degraded. The same *Mycobacterium* was reported by(Walter et al., 1991;Trzesicka & Ward, 1995). That Benzo(a)pyrene (Bap) has been degraded with other different bacteria including *Rhodococcus* sp., *Mycobacterium*, and mixed culture of Pseudomonas and Flavobacterium after 30 days incubation period.

Moreover, after 10 days *Mycobacterium confluentis* and bacteria F 6, 8 out of 13 compounds degraded. While bacteria DF all the compound were degraded except Carbamic acid, N-(2,3-dimethylphenyl)-, oxiranylmethyl ester at Retention Time (RT) 36.076 and (PA) 27.56% respectively and Methyl 2-methyl-2-(methoxy-3-hydroxypropoxy)amino-propanoate at Retention Time (RT) 40.275 and (PA) 14.75% respectively . *Mycobacterium confluentis* and bacteria F after 15 days all the compound were degraded. While bacteria DF was was degred except 3-Octene, (Z)- at (RT) 42.412 and Peak Area 11.14%. (Romero, Cazau et al. 1998) which described isolate *Pseudomonas aeruginosa* from a stream heavily polluted by a petroleum refinery. The species was found to be actively growing over high dosages of phenanthrene with complete removal of the pollutant in a period of 30 days.

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Table 2: Screening the by-products produced using GC-MS analysis from PAHs bioremediation at different incubation periods

NO	Control Compound & Molecular Formula	RT	Peak Area%	Bacterial Isolates and peak area (%) at different Incubation Period (day)								
				D			F			DF		
				5	10	15	5	10	15	5	10	15
1	Silane, 1,6-heptadiyne-1,7-diylbis [trimethyl $C_{13}H_{24}Si_2$	22.279	2.61	-	+ 5.25	-	-	-	-	+ 2.71	-	-
2	l-Methionine, n-heptafluorobutyryl-, isohexyl ester $C_{15}H_{22}F_7NO_3S$	25.629	4.70	-	+ 4.65	-	-	-	-	-	-	-
3	Adenosine, 2-methyl- $C_{11}O_4N_5H_{15}$	28.603	4.58	-	-	-	-	-	-	-	-	-
4	Benzoic acid, 4-[(trimethylsilyl)amino]-, trimethylsilyl ester $C_{13}H_{23}NO_2Si_2$	31.323	5.98	-	+ 7.87	-	-	+ 7.31	-	-	-	-
5	3-[2-Hydroxy-3-(3-hydroxy-phenylamino)-propyl]-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5] diazocin-8-one $C_{20}H_{25}N_3O_3$	33.789	7.56	-	+ 15.73	-	-	+ 7.55	-	+ 4.48	-	-
6	Carbamic acid, N-(2,3-dimethylphenyl)-, oxiranylmethyl ester $C_{12}H_{15}NO_3$	36.076	9.19	-	+ 13.29	-	-	+ 9.07	-	+ 10.84	+ 27.56	-
7	2-[4-Acetamidophenylsulfonyl]-1,4- naphthoquinone $C_{18}H_{13}NO_5S$	38.236	10.84	-	+ 15.52	-	-	-	-	+ 7.56	-	-
8	1,3,5-Triazine-2,4-diamine, N,N'-d iethyl $C_7H_{13}N_5$	39.287	6.13	-	-	-	-	-	-	-	-	-
9	Methyl 2-methyl-2-(methoxy-3-hydroxypropoxy)amino-propanoate $C_9H_{19}NO_5$	40.275	10.25	-	-	-	+ 4.93	-	-	-	+ 14.75	-
10	N-Benzyl-N-ethyl-p-isopropylbenzamide $C_{19}H_{23}NO$	42.186	7.13	-	-	-	-	+ 9.44	-	-	-	-
11	3-Octene, (Z)- C_8H_{16}	42.412	7.08	-	-	-	-	-	-	-	-	+ 11.14
12	2,2'-(2,2'-Oxybis(ethane-2,1-diyl) bis(oxy))bis(ethane-2,1-diyl) bis(3,5,5-trimethylhexanoate) $C_{26}H_{50}O_7$	43.220	20.54	-	-	-	-	-	-	-	-	-
13	trans-2,3-Methylenedioxy-b-methyl- b-nitrostyrene	43.965	3.41	+	+	-	-	+	-	+	-	-

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$C_{10}H_9NO_4$	3.45	3.63	2.89	6.35

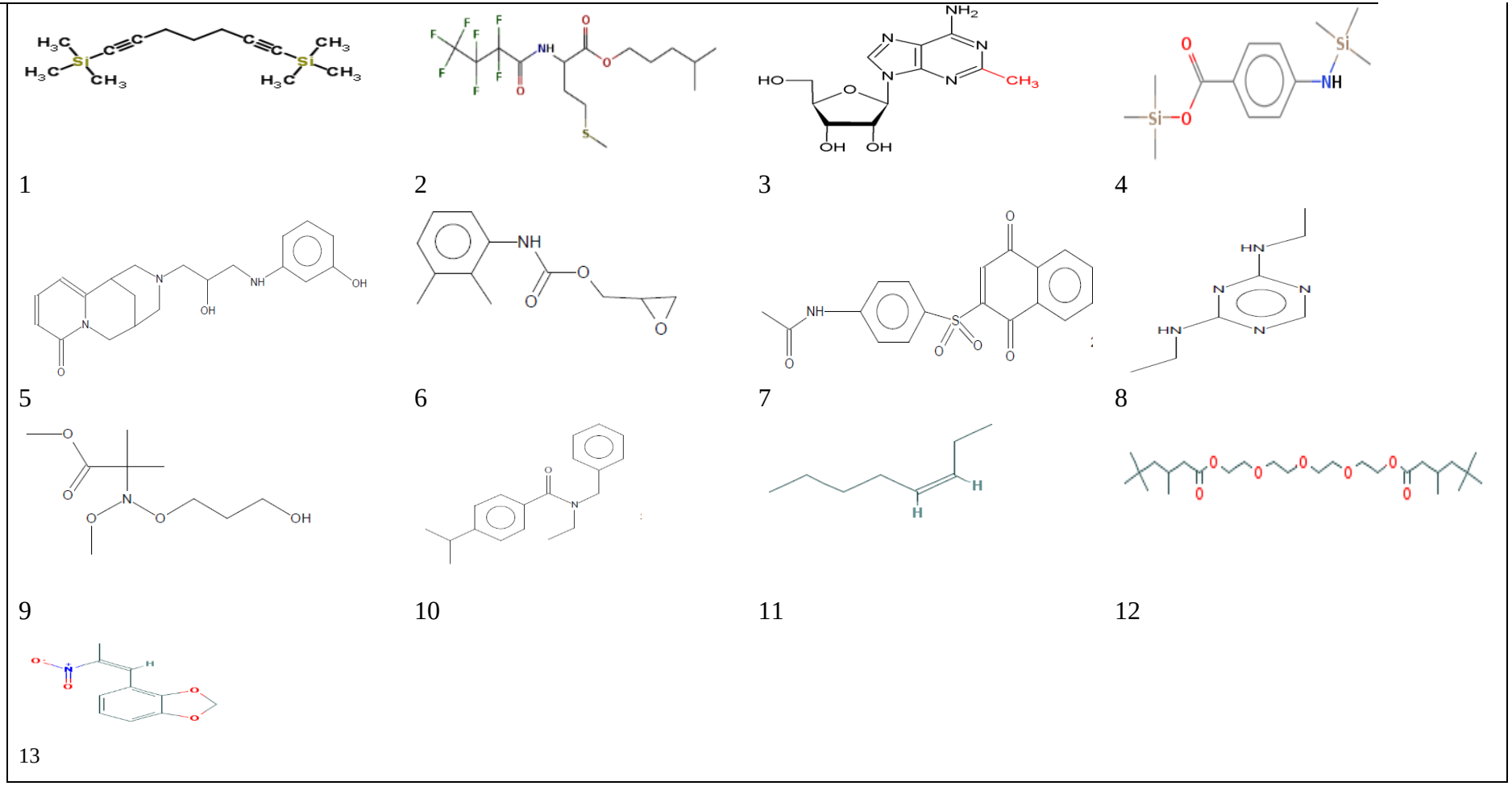

Fig 1: The major constituents compound structures of control before PAHs bioremediation.

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Data recorded in Table 3 revealed that GC-MS analysis confirmed new compound with medical and industrial importance after bacterial bioremediation of extracted PAHs. Fourteen compounds as Benzene, 1-nitro-3--(trifluoromethyl)-; Guanidine, N-methyl-N'-nitro-N-nitroso ; Hexadecanoic acid, methyl ester; dimethylcarbamothioic acid, O-isopropyl ester; Bis(2-ethylhexyl) phthalate; 3-buten-2-one, 4-(1-aziridinyl)-4-(dimethylamino); Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-; Fumaric acid, monoamide, N-(2methoxyphenyl)-, isohexyl ester; 4-Fluorohistamine; Morphinan, 7,8-didehydro-4,5-epoxy -17-methyl-3,6-bis[(trimethylsilyl)oxy]-, ; 3-Trimethylsilyloxystearic acid, trimethylsilyl ester; 9-Octadecenamide, (Z)-; 3-Amino-2-phenazinol ditms; Phenol, 2,5-bis(1,1-dimethylethyl)- with RT rang of (19.507-42.181).

After 5 days bacteria *Mycobacterium confluentis* and F were identified Guanidine, N-methyl-N'-nitro-N-nitroso as degradation products.While bacteria DF after 10 days. at Time (RT) 25.623 and Peak Area 1.38 , 2.88 , 20.42 % respectively. Inorder to understand Guanidines have been explored for wide ranging applications ranging explored for wide ranging applications ranging from materials to biology. The use of guanidines in nano-synthesis from materials to biology. The use of guanidines in nano-synthesis and dendrimer formation is an area yet to be explored and is an attractive future research perspective.Inaddition, known to be toxic are now identified as compounds with varied and numerous applications (Tahir et al., 2015).

Moreover after 15 days Bis(2-ethylhexyl) phthalate, synonym is (Phthalic acid, bis(2-ethylhexyl) ester, DEHP was identified as degradation in bacteria *Mycobacterium confluentis* , F and DF at Time (RT) 39.287 and Peak Area 57.44 , 60.87 ,40.13 % respectively. In addition ,after 5,10 days in *Mycobacterium confluentis* and F at Peak Area 29.94 , 8.55 % respectively. The same *Mycobacterium* , product and metabolized 61.5 % after 5 days were reported by (Rehmann et al., 1998) isolated a Mycobacterium spp., strain KR2 from a PAH contaminated soil of a gaswork plant, which was able to utilize pyrene as sole source of carbon and energy. The isolate metabolized up to 60% of the pyrene added (0.5mgml−1) within 8 days at 20 ◦C. Cis-4,5-pyrene dihydrodiol, 4-5-phenanthrene dicarboxylic acid, 1- hydroxy-2-naphthoic acid, 2-carboxybenzaldehyde, phthalic acid, and protocatechuic acid were identified as degradation products. Due to its suitable properties and the low cost, DEHP is widely used as a plasticizer in manufacturing of articles made of PVC.

Another compounds with application Fumaric acid, monoamide, N-(2methoxyphenyl)-, isohexyl ester and Phenol, 2,5-bis(1,1-dimethylethyl)- in bacteria *Mycobacterium confluentis* and F after 15 days at at Time (RT) 28.950 and Peak Area 1.60 , 3.06 % respectively.while Phenol, 2,5-bis(1,1-dimethylethyl)- at (RT) 19.507 and Peak Area 6.66 , 13.91 % respectively . Currently, phenol compounds are the most common organic pollutants presenting wastewater due to the continuing process of industrialization. These compounds have been used extensively used extensively in various industries, such as the used extensively in various industries ,such as the chemical and petrochemical industries (Hasanoğlu 2013;Naeem & Ouyang 2013).

Hexadecanoic acid, methyl ester (palmitic acid) showed after 5 days by bacteria F at (RT) 28.742 and Peak Area 52.31 %. While Morphinan, 7,8-didehydro-4,5-epoxy -17-methyl-3,6-bis[(trimethylsilyl)oxy]-, was degreded by bacteria DF at (RT) 25.623 and Peak Area 4.18 %. (Tewari, Jyothi et al. 2015) described specific application of hexadecanoic ester derivatives in

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Table 3: New compound produced with industrial importance by-products using GC-MS from PAHs Bioremediation at different incubation periods

NO	Compounds produced and molecular formula	RT[1]	Bacterial isolates and peak area (%) at different incubation Period(day)									Application
			D			F			DF			
			5	10	15	5	10	15	5	10	15	
1	Benzene, 1-nitro-3--(trifluoromethyl)- $C_7H_4F_3NO_2$	19.513	+[2] 1.58[4]	-[3]	-	+ 3.55	-	-	+ 1.55	-	-	industrial solvent in paints
2	Guanidine, N-methyl-N'-nitro-N-nitroso $C_2H_5N_5O_3$	25.623	+ 1.38	-	-	+ 2.88	-	-	-	+ 20.42	-	multiple biological applications- uses of chemistry
3	Hexadecanoic acid, methyl ester(palmitic acid $C_{17}H_{34}O_2$	28.742	-	-	-	+ 52.31	-	-	-	-	-	Medical- industrial effect of insct- industrial applications
4	Dimethylcarbamothioic acid, O-isopropyl ester $C_6H_{13}NOS$	38.236	+ 0.85	-	-	+ 6.14	-	-	-	-	-	
5	Bis(2-ethylhexyl) phthalate $C_{24}H_{38}O_4$ (.Phthalic acid, bis(2-ethylhexyl) ester)	39.287	+ 29.94	-	+ 57.44	-	+ 8.55	+ 60.87	-	-	+ 40.13	industrial
6	3-buten-2-one, 4-(1-aziridinyl)-4-(dimethylamino) $C_8H_{14}N_2O$	28.782	-	-	+ 2.10	-	-	+ 4.75	-	-	+ 4.97	
7	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]- $C_{12}H_{36}O_4Si_5$	42.181	+ 7.59	-	-	-	+ 6.02	-	-	-	-	
8	Fumaric acid, monoamide, N-(2methoxyphenyl)-, isohexyl ester $C_{17}H_{23}NO_4$	28.950	-	-	+ 1.60	-	-	+ 3.06	-	-	-	Food-medicine-psoriasis
9	4-Fluorohistamine $C_5H_8FN_3$	29.400	-	-	-	-	-	+ 4.25	-	-	+ 7.77	medical
10	Morphinan, 7,8-didehydro-4,5-epoxy -17-methyl-3,6-bis[(trimethylsilyl)oxy]-, $C_{23}H_{35}NO_3Si_2$	25.623	-	-	-	-	-	-	+ 4.18	-	-	medical
11	3-Trimethylsilyloxystearic acid, trimethylsilyl ester $C_{24}H_{52}O_3Si_2$	40.281	+ 11.16	-	-	-	-	-	+ 15.99	-	-	Steel, petrochemical, coal chemical, Fertilizer, metallurgy, Electricity
12	9-Octadecenamide, (Z)- , Oleic acid amide $C_{18}H_{35}NO$	18.427	-	+ 2.29	-	-	-	-	+ 1.42	-	-	Industry uses - Consumer Uses
13	3-Amino-2-phenazinol ditms $C_{18}H_{25}N_3OSi_2$	22.85	-	-	-	-	+ 5.17	-	+ 7.01	-	-	
14	Phenol, 2,5-bis(1,1-dimethylethyl)- $C_{14}H_{22}O$	19.507	-	-	+ 6.66	-	-	+ 13.91	-	-	-	the chemical and petrochemical industries

RT: Retention time; +[2]: Compound Exist; -[3]: Compound absent; [4]: peak Area %.

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traps for effective management of this groundnut pest. The specificity of synthesized ester derivatives in attracting both sexes differently also indicates their probable resemblance to pheromone components of *Caryedon serratus* . Moreover, Palmitic acid has also a wide range of industrial applications, including the use in the production of soaps and cosmetics as well as being utilized as a food additive.(Albuquerque et al., 2005;Simakova et al., 2009).While according (Schuster, Spetea et al. 2010) were reported that presents a large investigation of morphinan and isoquinoline compounds as AChE inhibitors. Pure cultures of microbes are capable of metabolizing only a certain range of PAHs due to their complex structure. Therefore, several microbial species are assembled to form microbial consortium with broad enzymatic capacities to increase the rate and degradation of PAHs. Such mixed cultures display metabolic versat ility and superiority to pure cultures and superiority to pure cultures (Gupta et al., 2016).

Fumaric acid used effective treatment of psoriasis has been reported by (van Geel et al., 2016). This compound found when degradation after 15day by *Mycobacterium confluentis* and bacteria F at (RT) 28.950 and Peak Area 1.60 ,3.06 %respectively. Some bacteria like Mycobacterium sp. are capable of oxidizing PAHs by the action of the cytochrome P450 monoxygenase enzyme to form trans-dihydrodiols (Kelley et al., 1990).

3-Trimethylsilyloxystearic acid, trimethylsilyl ester, 9-Octadecenamide, (Z)-,(Oleic acid amide), 3-Amino-2-phenazinol ditms were found after 5 days by bacteria DF at (RT) 40.281,18.427,22.85 and Peak Area 15.99 ,1.42,7.01 %respectively. Several bacterial species such as Pseudomonas alcaligenes, Rhodococcus sp., Mycobacterium sp., Sphingomonas sp. are found to be capable of degrading PAHs. Most of these bacteria have the capability to grow on low molecular PAHs such as napthalene, fluorene and phenanthrene. But in last few years several bacteria (especially Mycobacterium) have been isolated to grow on four ring PAHs. The hydrophobic surfaces of these bacteria help them to adhere to hydrophobic PAHs, leading to their mass transfer inside the cell (Seo et al., 2009).

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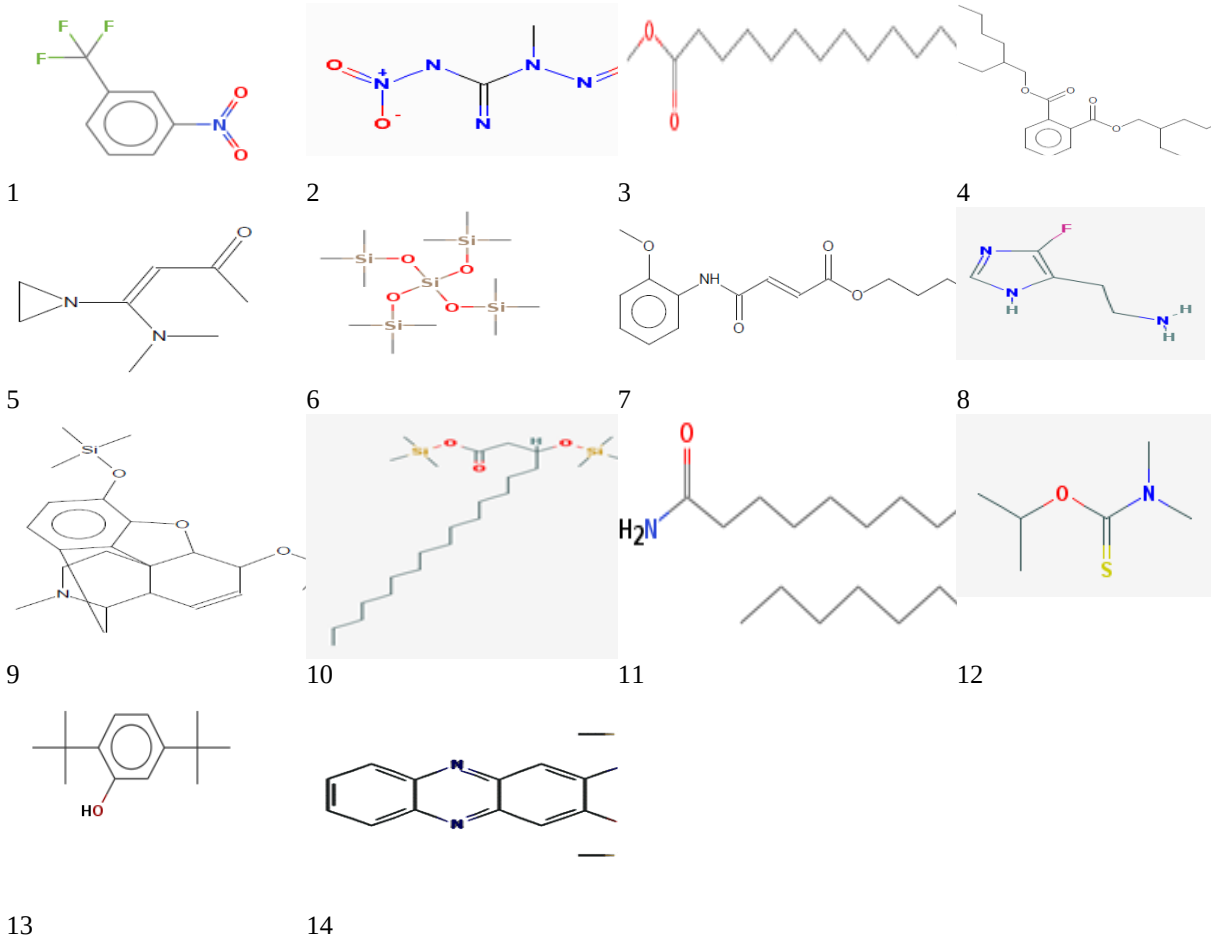

Fig 2: The major constituents compound structures produced from bioremediation PAHs

CONCLUSIONS

Two bacteria have been isolated from contaminated water and applied for PAHs degradation, hence produce new compounds with highly medical and industrial importance. All the compound were degraded after 15 days. Further research was needed to explore the within the PAH-degrading *Mycobacterium confluentis*, bacteria F and DF and mechanisms involved during the biodegradation of low and high-molecular weight PAHs.

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