INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 3, p. 335-349, 2017

RESEARCH PAPER OPEN ACCESS

Isolation, screening and adaptation of bacteria isolated from sewage waste water and their ability to degrade pyrene through co-metabolism

Nadia Bibi*

Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan

Key words: PAHs, Solid media screening, Spray plate technique, Co-metabolism, Sewage waste water

http://dx.doi.org/10.12692/ijb/10.3.335-349 Article published on March 31, 2017

Abstract

Eleven bacterial wild strains were isolated from sewage waste water of PCSIR Laboratory Colony Peshawar, through serial dilution and were adapted for degradation of polycyclic aromatic hydrocarbons (PAHs). These isolates were screened for low molecular weight (LMW) and high molecular weight (HMW) PAHs. Isolated strains were screened on solid nutrient agar and then on mineral media. These bacterial isolates can bring about complete mineralization of (HMW) like pyrene. Isolates W_1, W_2, W_8 and W_{11} were best on pyrene and phenanthrene respectively. PAHs compounds produced yellow coloration on mineral media plates. This yellow coloration during degradation of PAHs is the characteristic property of bacterial conversion of polyaromatic hydrocarbons to 2-hydroxylmuconic semi-aldehyde through meta-cleavage of catechol. Among these isolates, only W_2 was able to grow on pyrene individually and efficiently degraded PAHs mixture. Naphthalene and acenaphthene were completely degraded in 24 hours as revealed from HPLC analysis. Catechol (64.05%) and phthalic acids (31.44%) were detected after 72 hours are the degradation products of phenanthrene and anthracene. 1-hydroxy2-naphthoic acid (63.37%) is the oxidation products of phenanthrene through naphthalene degradation pathway. Increased production of benzocaumarin (32.89-93.13%) between incubation periods of 120-168 hours can be the possible explanation that selected PAHs present in the mixture were degraded through naphthalene pathway. Experiment was performed in triplicate and data was analysed by 2-way ANOVA and level of significance < 0.002.

* **Corresponding Author:** Nadia Bibi ✉ botanist_gemini@yahoo.com

Introduction

Many PAHs are in the United States Environmental Protection Agency Priority Pollutant List, including acenaphthene, naphthalene, anthracene, phenanthrene and pyrene due to their toxicity, carcinogenecity and teratogenecity (Lily *et al.*, 2013). Various sources of PAHs production are mainly from domestic and industrial effluents and sewage waste waters from agriculture sources and hospitals. From there these PAHs compounds gets entry into canals and rivers and impact the aquatic environment by targeting the bottom feeder such as polycaetes, nematode, bivalves and crustaceans (Smith *et al.*, 2007; Moreno *et al.*, 2009), then enter the food chain which is an important pathway of entry into human bodies.

Waste water irrigated land accumulates huge quantities of these contaminants and also reach the green parts of plants. Detection of PAHs in soil and green leaves vegetable in different areas metropolitan areas of Khyber Pakhtunkhwa (223-929mgkg^{-1} in soils & 51.6-402mgkg^{-1}) as revealed from quantification studies (Waqas *et al.*, 2014). It can be concluded that the waste water contains these contaminants in huge quantities. The incidence of Tasman spirit in July 2003 adds a lot of oil to the coastal beach of Clifton Karachi. This oil spill affected the phytoplankton in the coastal area of Karachi, and the people living there were observed with different problems like low efficiency of lungs and gastro intestinal tract, itching and headache (Saifullah and Chaghtai, 2005; Janjua *et al.*, 2006). The major concern is about PAHs which act as ubiquitous pollutant with potential toxic and carcinogenic effect, persist in the environment for longer period due to their hydrophobic nature. The genotoxicity of PAHs also increases with their size, up to at least four or five fused benzene rings. Release of toxic PAHs by natural and anthropogenic activities into the ecosystem is the root cause for air, water and soil pollution which leads to deleterious effects on plants, animals and human health (Menzie *et al.*, 1992; Yuan *et al.*, 2000).

These contaminants can be removed by various physical means but form more hazardous compounds than the parents one (Soojhawon *et al.*, 2005; Hassan *et al.*, 2009). Co-metabolic bioremediation is the breakdown of a contaminant by an enzyme or cofactor that is formed during microbial metabolism of another compound. This type of bioremediation is highly targeted because it ensures that only microbes that can degrade the contaminant of concern are stimulated. Hence, these microorganisms cannot get any carbon and energy benefit (Bastiaens *et al.*, 2000; Sipkema *et al.*, 2000; Rui *et al.*, 2004; Semprini *et al.*, 2005; Hazen *et al.*, 2010; Powell *et al.*, 2011). The interactions between different microorganisms under mixed culture conditions such as co-metabolism might be important too, and biodegradation of toxic organic compounds by the mixed culture could be different from that of a single culture (Kobayashi and Ritmann, 1982; Tam *et al.*, 2002). It is a common practice that bacteria isolated from non-contaminated sediments can be adapted to the contaminated environment (Chaineau *et al.*, 1999). In addition to the initial PAH concentration, the addition of a simple carbon source such as glucose might also influence the biodegradation potential. In present study eleven bacterial strains were isolated from sewage waste water and screened on solid media for utilization of the five selected PAHs individually as well as in mixture. To the best of our knowledge this is first reported work on bacterial degradation of PAHs isolated from sewage waste water of Peshawar and therefore aims (1) to investigate the biodegradation potential of the bacterial isolates from sewage waste water to degrade PAHs on solid media (2) to study degradation of PAHs mixture by these bacteria through co-metabolism.

Materials and methods

Chemicals and Media

Nutrient agar and Mineral salt medium was used for initial screening. The composition of PNR and PNRG (PNR + 5 mM glucose) per liter of distilled water (Khan *et al.*, 2006; Survery *et al.*, 2009), is as; PN (20x) 50mL used as 50 mlL^{-1}: KH_2PO_4 13.6% (wv^{-1}), $(NH_4)_2SO_4$ 2.4% (wv^{-1}), NaOH 2.5% (wv^{-1}) and R salts used as 7mlL^{-1} $MgSO_4.7H_2O$ 8% (wv^{-1}), $FeSO_4.7H_2O$ 0.2% (wv^{-1}), HCl 0.4 % (wv^{-1}), Agar (2%) was used as solidifying agent. All the chemicals naphthalene, acenaphthene, anthracene, phenanthrene, pyrene,

salicylic acid, salicylaldehyde, dicarboxylic acid and catechol, purchased from Merck and Fluka and were analytical grade.

Collection of soil samples

Sewage waste water of PCSIR Laboratory Colony Peshawar was collected. The samples were placed into sterile tubes and immediately brought to the laboratory and stored at 4°C.

Isolation of bacteria from sewage waste water

Bacteria were isolated from the water samples using serial dilution and spread plate technique on nutrient agar media and incubation till appearance of growth. Inoculated plates were purified by repeatedly by sub-culturing and streak plate method. Colonies appeared were picked and cultured in new nutrient agar. Multi steps of purification were performed until pure strains were obtained. Purification process involved streak plate method was done to ensure only single strain was obtain for each plating. Pure cultures obtained were stored in slants of enrichment medium with 2.0gL^{-1} pure agar and stored at 4°C (Survery *et al.*, 2009).

Screening on solid media for PAHs degradation

Screening of the isolates for the ability to use anthracene as sole source of carbon and energy for biodegradation was carried out on both solid and liquid mineral media. A qualitative assay by the spray-plate method was used to check the degradation potential of the isolated bacterial strains for growth on PAHs (Kiyohara *et al.*, 1982; Khan *et al.*, 2010; Kumar *et al.*, 2010; Alias *et al.*, 2011; Dastgheib *et al.*, 2011). Initially, the strains were screened on nutrient agar and then on mineral salt medium (PNRG) and (PNR). Initially, the strains were screened on nutrient agar and then on mineral salt medium (PNRG) and (PNR). Initial concentration range was 25 ppm-1200 ppm for anthracene, 100-800ppm (phenanthrene), 100-1000ppm (naphthalene and acenaphthene), 10-1000ppm (pyrene) and incubated until appearance of growth. PNR plates were prepared and inoculated with bacteria and sprayed with 0.1% of all the five selected PAHs which was appropriately labeled. The plates were incubated at room temperature (about 28°C) for four days. Appearance of growth on mineral plates with PAHs spray was an indication of the utilization of PAHs. Those strains with best growth were selected for further studies. To confirm the degradation capability, PAHs were added to liquid mineral media as sole carbon and energy source. To confirm the degradation capability, PAHs were added to liquid mineral media as sole carbon and energy source. Co-metabolism study was performed according to protocol of (Zhong *et al.*, 2007) with some modifications.

Inoculums preparation

All the isolates were grown in Nutrient Broth and incubated at 150 rpm in an orbital shaker at 28±2°C. After reaching late exponential phase bacterial cells were harvested at 8000×g at 4°C for 10 minutes, washed twice in phosphate buffer (pH 7.2) and resuspended in BSM to obtain bacterial suspension with an OD 600nm less than 0.4. The suspension was used as the inoculums in all optimization, biodegradation and co-metabolism experiments (Guo *et al.*, 2010).

Co-metabolism experiment

Experiment for cometabolic degradation of mixed PAH, and metabolites were studied on the basis of retention time comparison with available authentic standards and literature values. Experiment for cometabolic degradation of five selected compounds was prepared according to established protocol with some amendments (Zhong *et al.*, 2007). Before bacterial inoculation, the added PAH was evenly mixed with the liquid medium, and the flasks were shaken on a rotary shaker (150 rpm) at 30°C overnight. The added acetone was evaporated, and the residual acetone was so small that it did not have any toxic effect to microorganisms according to our previous experience. The isolate was grown in nutrient broth (NB) for 3 days, centrifuged at 5,000×g for 10 min, washed twice, and resuspended in 0.85% NaCl to obtain the cell suspension with an optical density at OD (600_{nm}) of 1.0. The 500-µl aliquot of the cell suspension previously grown in NB was used as an inoculum and was added to the respective growth medium.

A sterilized PAH flask without any bacterial inoculation was used as the abiotic control. Each treatment and control was prepared in triplicate. All cultures were started with cell density at an OD of 0.02 and were incubated at 30°C on a rotary shaker (150 rpm) in the dark. The growth in terms of OD of the culture was measured at days 0, 1, 3, 5, and 7.

Extraction of selected PAHs from sample

The samples were then agitated in an incubated shaker at 150rpm for two weeks. Samples were periodically collected from reactor flasks for day 1, 2, 5, 7 for the purpose of measuring residual concentration of PAHs. PAHs were extracted by protocol of Shokrollahzadeh *et al* with some modifications (Supakaa *et al*., 2001). The growth medium was extracted twice by dichloromethane (DCM) and acetone 10mL each by shaking at 30°C for 24 hours. The extraction phase was stripped of water droplets by pipetting the upper layer after centrifugation at 12,000xg. The remaining moisture was removed by addition of 4gm anhydrous sodium sulphate (Na_2SO_4) and evaporated to dryness in a rotary evaporator and concentrated in a rotary evaporator to around 2ml. The samples were then filtered, using 0.45 µm syringe filters.

High performance liquid chromatography (HPLC) analysis

Standard protocol was followed for extraction of anthracene metabolites for HPLC analysis (Shokrollahzadeh *et al*., 2012). Metabolites were studied on the basis of retention time comparison with available authentic standards and literature values. Followed by the extraction with ethyl acetate, the samples were filtered through 0.2mm syringe filter and analyzed in a high performance liquid chromatography.

HPLC (PerkinElmer) with the C18 reverse phase column was used to analyze anthracene under isocratic condition using acetonitrile: water (80:20) (vv^{-1}) as mobile phase and detection wavelength –254 nm. The flow rate of the mobile phase (acetonitrile) was maintained at 1.2mLmin^{-1}. The samples volume was 2mL in each vial of HPLC tray. Metabolites were studied on the basis of retention time comparison with available authentic standards and literature values.

Results

Isolation of bacteria from sewage waste water

In present study a total of 11 bacterial strains were isolated from sewage waste water were isolated. Isolation was done through serial dilution and spread plate techniques in spite of classical enrichment. These wild isolates after repeated purification steps were used for initial screening on selected PAHs individually and in mixture.

Screening of LMW PAHs (anthracene, naphthalene phenanthrene and acenaphthene) biodegrading bacteria isolated from sewage waste water

We isolated 11 bacteria (W-strains) from sewage water and grow it on anthracene amended nutrient agar media as shown in Table 1. The concentration range for anthracene was (25-1200ppm), the resistant strains were processed on other selected PAHs as well. These isolates when tested on naphthalene three isolate show concentration dependent growth upto 800ppm and two were best at 1100ppm as shown in Table 2. Six isolates were best on 700ppm among which two show rich growth and three with moderate growth rate on phenanthrene and acenaphthene at (1100ppm) as clear from Table 3 & 4.

Table 1. Screening of W-strains on anthracene amended nutrient agar media.

S. No	Isolates	Anthracene concentration in ppm													
		25	50	100	200	300	400	500	600	700	800	900	1000	1100	1200
1.	W_1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2.	W_2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3.	W_3	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	W_4	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+
5.	W_5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	-	-
6.	W_6	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	+	-	-
7.	W_7	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	-

S. No	Isolates	Anthracene concentration in ppm													
		25	50	100	200	300	400	500	600	700	800	900	1000	1100	1200
8.	W_8	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	-	-	-
9.	W_9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	-	-
10.	W_{10}	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	+
11.	W_{11}	+++	+++	+++	++	++	++	++	++	++	++	+	+	+	-

+++ = Rich growth ++ = Medium growth + =Less growth (-) = No growth.

Table 2. Screening of W-strains on naphthalene amended nutrient agar media.

S. No	Isolates	Naphthalene concentration in ppm											
		50	100	200	300	400	500	600	700	800	900	1000	1100
1.	W_1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2.	W_2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3.	W_3	+++	+++	+++	+++	++	++	++	++	+	+	-	-
4.	W_4	+++	+++	+++	+++	+++	+++	+++	+++	+	-	-	-
5.	W_5	+++	+++	+++	+++	+++	+++	+++	++	++	++	+	-
6.	W_6	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	-
7.	W_7	+++	+++	+++	+++	+++	+++	++	++	+	+	-	-
8.	W_8	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++
9.	W_9	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
10.	W_{10}	+++	+++	+++	+++	+++	+++	+++	++	++	++	+	+
11.	W_{11}	+++	+++	++	++	++	++	++	++	++	++	++	++

+++ = Rich growth ++ = Medium growth + =Less growth (-) = No growth.

Table 3. Screening of W-strains on phenanthrene amended nutrient agar media.

S. No	Isolates	Phenanthrene concentration in ppm											
		50	100	200	300	400	500	600	700	800	900	1000	1100
1.	W_1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2.	W_2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3.	W_3	+++	+++	+++	+++	++	++	++	++	+	+	-	-
4.	W_4	+++	+++	+++	+++	+++	+++	+++	+++	+	-		-
5.	W_5	+++	+++	+++	+++	+++	+++	+++	++	++	++	+	-
6.	W_6	+++	+++	+++	+++	+++	+++	+++	++	+	-	-	-
7	W_7	+++	+++	+++	+++	+++	+++	++	++	+	+	-	-
8	W_8	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++
9	W_9	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
10	W_{10}	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+
11.	W_{11}	+++	+++	++	++	++	++	++	++	++	++	++	++

+++ = Rich growth ++ = Medium growth + =Less growth (-) = No growth.

Table 4. Screening of W-strains on acenaphthene amended nutrient agar media.

S. No	Isolates	Acenaphthene concentration in ppm											
		50	100	200	300	400	500	600	700	800	900	1000	1100
1.	W_1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2.	W_2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3.	W_3	+++	+++	+++	+++	++	++	++	++	+	+	-	-
4.	W_4	+++	+++	+++	+++	+++	+++	+++	+++	+	-		-
5.	W_5	+++	+++	+++	+++	+++	+++	+++	++	++	++	+	-
6.	W_6	+++	+++	+++	+++	+++	+++	+++	++	+	+	-	-
7.	W_7	+++	+++	+++	+++	+++	+++	++	++	+	+	-	-
8.	W_8	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++
9.	W_9	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
10.	W_{10}	+++	+++	+++	+++	+++	+++	+++	++	++	++	+	+
11.	W_{11}	+++	+++	++	++	++	++	++	++	++	++	++	++

+++ = Rich growth ++ = Medium growth+ =Less growth (-) = No growth.

Screening of LMW PAHs (pyrene) biodegrading bacteria isolated from sewage water

Results of W- isolates on pyrene amended media as shown in Table 5. Eight of these isolates showed rich and two with moderate growth rate up to 600ppm as shown in Table 5. Three of these isolates were resistant at 1000ppm.

Adaptaion of bacterial isolates for enhanced degradation

Microbes can be adapted for biodegradation of PAHs through changing concentration and growth media i.e. from simple nutrient agar to mineral media with (PNRG) and without glucose (PNR) as shown in Table 6 and 7.

Screening in liquid media for selection of best degraders

Results of the isolates for confirmation of growth on PAHs are shown in Table 8. The results of growth of W-isolates on selected PAHs individually after 72hours of incubation are shown in following section:

Table 5. Screening of W-strains on pyrene amended nutrient agar media.

S. No	Isolates	Pyrene concentration in ppm												
		10	20	50	100	200	300	400	500	600	700	800	900	1000
1.	W_1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2.	W_2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3.	W_3	+	-	-	-	-	-	-	-	-	-	-	-	-
4.	W_4	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+
5.	W_5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	-
6.	W_6	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	+	-
7.	W_7	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+
8.	W_8	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	-	-
9.	W_9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	-
10.	W_{10}	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
11.	W_{11}	+++	+++	+++	++	++	++	++	++	++	++	+	+	+

+++ = Rich growth ++ = Medium growth + =Less growth (-) = No growth.

Table 6. Adaptation of bacterial isolates on selected PAHs amended PNRG media.

S. No.	Isolates	PAHs Conc. (ppm) /PNRG				
		Acenaphthene	Naphthalene	Anthracene	Phenanthrene	Pyrene
		100-900	100-1000	50-1200	100-1100	10-1000
1.	W_1	+++	+++	+++	+++	+++
2.	W_2	+++	+++	+++	+++	+++
3.	W_5	+++	+++	+++	+++	+++
4.	W_8	+++	+++	+++	+++	+++
5.	W_9	+++	+++	+++	+++	+++
6.	W_{10}	+++	+++	+++	+++	+++
7.	W_{11}	+++	+++	+++	+++	+++

Table 7. Adaptation of selected bacterial isolates on selected PAHs compounds amended PNR media.

S. No.	Isolates	PAHs Conc. (ppm) /PNR				
		Acenaphthene	Naphthalene	Anthracene	Phenanthrene	Pyrene
		100-900	100-1000	50-1200	100-1100	10-1000
1.	W_1	+++	+++	+++	+++	+++
2.	W_2	+++	+++	+++	+++	+++
3.	W_5	+++	+++	+++	+++	+++
4.	W_8	+++	+++	+++	+++	+++
5.	W_9	+++	+++	+++	+++	+++
6.	W_{10}	+++	+++	+++	+++	+++
7.	W_{11}	+++	+++	+++	+++	+++

Table 8. Screening of W-isolates in liquid media under static lab conditions.

S. No	Isolates	PAHs/ OD 600nm				
		Anthracene	Naphthalene	Phenanthrene	Acenaphthene	Pyrene
1.	W_1	0.09	0.101	0.210	0.110	0.826
2.	W_2	0.290	0.185	0.180	0.123	0.494
3.	W_5	0.139	0.158	0.126	0.110	0.336
4.	W_8	0.110	0.167	0.121	0.131	0.600
5.	W_9	0.170	0.125	0.101	0.114	0.253
6.	W_{10}	0.258	0.120	0.112	0.121	0.284
7.	W_{11}	0.282	0.07	0.668	0.257	0.327

Growth of W-isolates on selected PAHs individually in liquid mineral media

Growth of isolate W_1 on the selected PAHs is shown in Fig.1. The highest growth OD value of 0.826 was observed on pyrene and 0.210 on phenanthrene. Growth of isolate W_2 on the selected PAHs is shown in Fig.2. In case of isolate W_2 on the growth OD was 0.494 on pyrene and 0.290 on anthracene. Isolate W_5 on pyrene give a growth OD value of 0.336 among the selected PAHs is shown in Fig. 3. Highest growth was recorded for isolate W_8 on pyrene with an OD value of 0.600 among the selected PAHs is shown in Fig.4. The growth of isolate W_9 on the selected PAHs was not significant as clear from the graph the lower OD values shown in Fig.5. Growth of isolate W_{10} on the selected PAHs were smaller as shown in Fig.6. Growth of isolate W_{11} was best on phenanthrene with OD value of 0.668 following by pyrene with 0.327 and lower for anthracene and acenaphthene with 0.258 and 0.257 respectively as shown in Fig. 7. If we compare the growth pattern of these isolates on individual PAHs compounds, it is clear from the graph (Fig. 8) that W_1, W_2 & W_8 were best on pyrene and W_{11} on phenanthrene. These isolates were selected for co-metabolism studies on the basis of best growth on single PAHs.

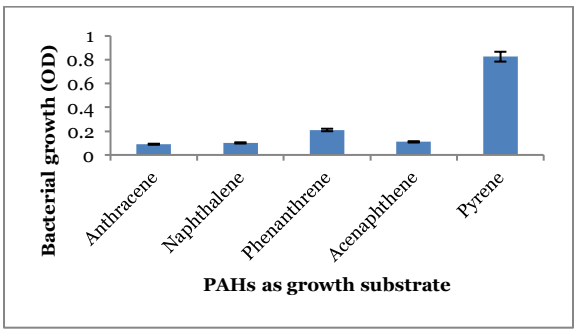

Fig. 1. Growth of isolate W_1 on selected PAHs in 72 hours.

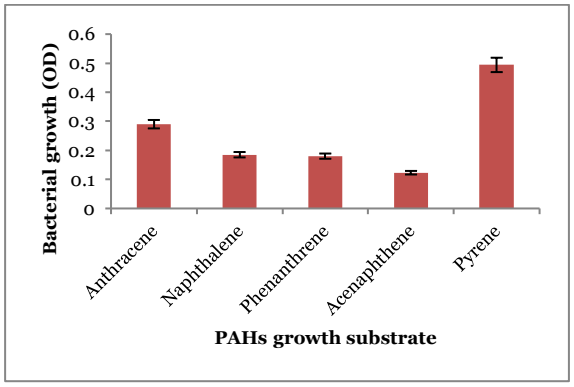

Fig. 2. Growth of isolate W_2 on selected PAHs in 72 hours.

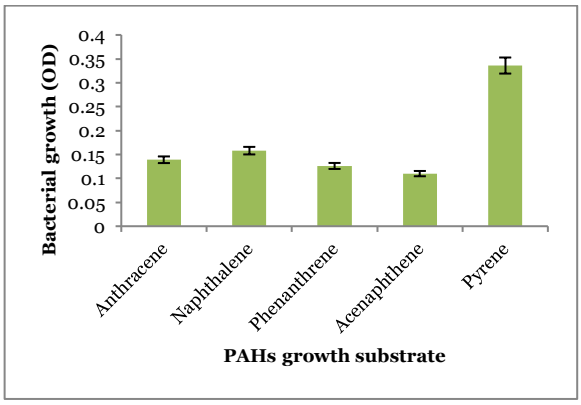

Fig. 3. Growth of isolate W_5 on selected PAHs in 72 hours.

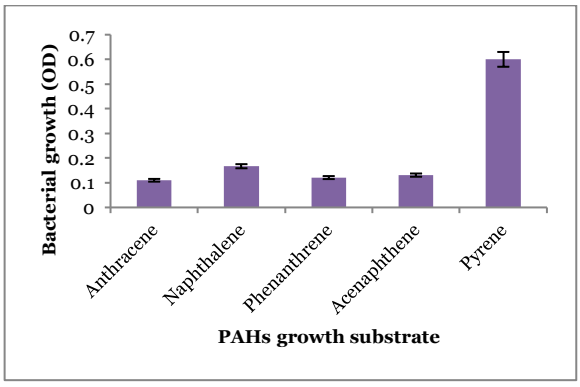

Fig. 4. Growth of isolate W_8 on selected PAHs in 72hrs.

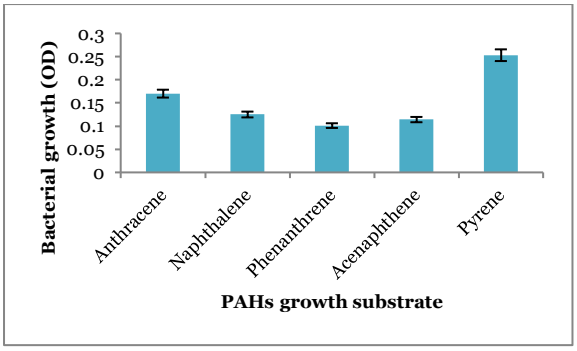

Fig. 5. Growth of isolate W_9 on selected PAHs in 72hrs.

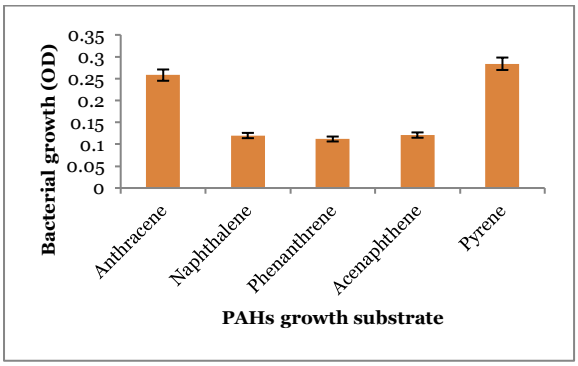

Fig. 6. Growth of isolate W_{10} on selected PAHs in 72hrs.

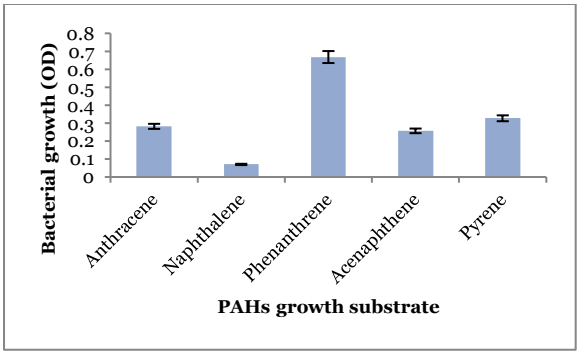

Fig. 7. Growth of isolate W_{11} on selected PAHs in 72hrs.

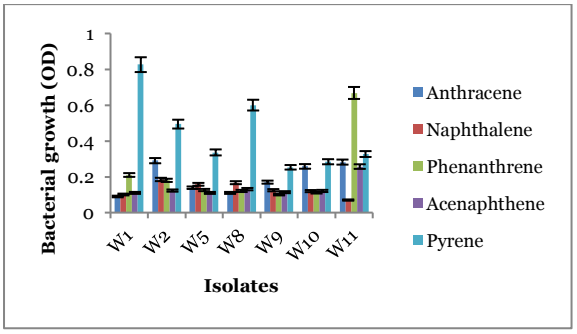

Fig. 8. Comparison of growth of W-isolates on five selected PAHs individually after 72 hrs.

Growth of isolates on mixture of selected PAHs

The isolates with best growth on individual PAHs were subjected to mixture of PAHs as shown in Fig.9. isolate W_1 showed very slow growth rate with an OD value of 0.225 after 168 hours of incubation. Maximum growth rate of isolate W_8 on mixture of PAHs was observed after 72 hours with OD of 0.252 as shown in Fig.10. On further incubation growth OD values drop to 0.229 and 0.072 after 120 and 168 hours respectively. Growth OD value was smaller for isolate W_{11} on mixture of PAHs as shown in Fig.11. The growth of isolate W_2 was highest on mixture of selected PAHs (Fig.12) and this isolate was selected for the further co-metabolism studies.

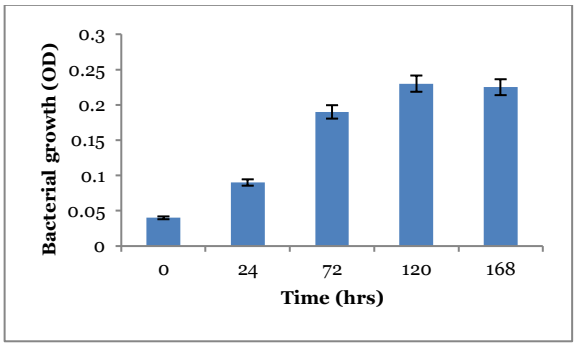

Fig. 9. Growth of isolate W_1 on mixture of PAHs.

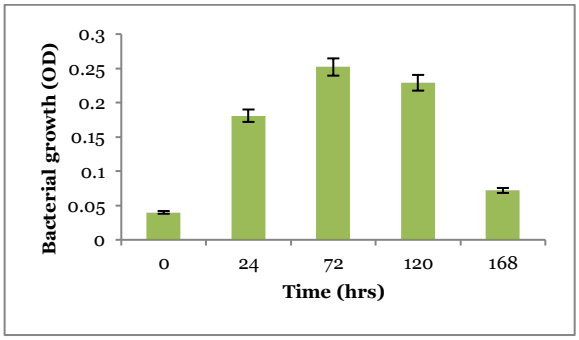

Fig. 10. Growth of isolate W_8 on mixture of PAHs.

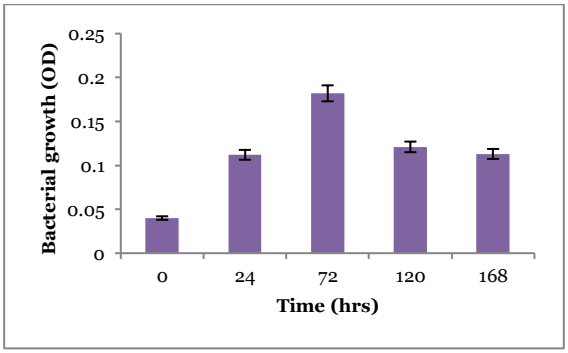

Fig. 11. Growth of isolate W_{11} on mixture of PAHs.

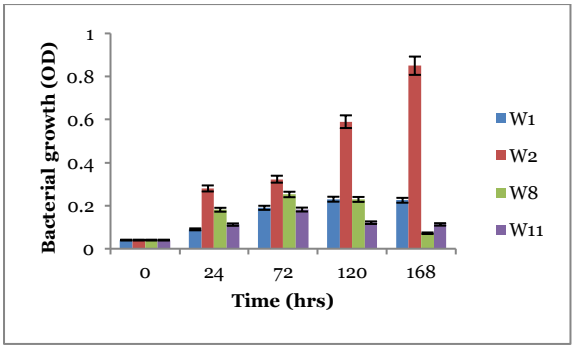

Fig. 12. Comparison of growth pattern of isolates on mixture of PAHs.

Co-metabolic degradation by isolate W_2

The growth pattern of isolate W_2 on mixture of PAHs is shown in Fig.13. The growth rate was a bit slower between incubation period of 0-72hours. However, a two-three fold increase in growth rate was detected between 72hours with growth OD's of 0.590 (after 120hours) and 0.85 (after 168hours). The list of metabolites formed by isolate W_2 analyzed through HPLC on comparison with authentic standards is summarized in Table 9.

Table 9. Metabolites observed during HPLC analysis for isolate W_2 during co-metabolism.

Time in Hours	HPLC Metabolites
24	Pyrenedihydrodiol 65.91% and 1-methoxy-2,hydroxyanthracene 29.73%
72	Catechol 64.05% and phthalic acids 31.44%
120	Benzocaumarin 32.89% and 1-hydroxy2-naphthoic acid 63.37% Phthalic acid 32.03% and catechol 63.77%
168	Benzocaumarin 93.13% and traces of pyrene metabolites

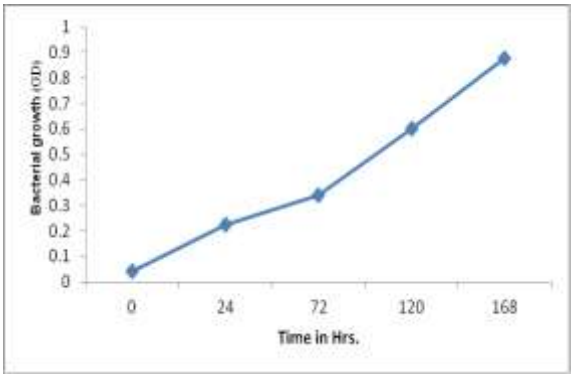

Fig. 13. Growth of W_2 on mixture of five PAHs.

Discussion

Isolation and use of microorganism from the rhizospheric soil has long been in practice and are giving good results. But, sedimentation of PAHs in water bodies due to their persistence, researchers are now been targeting the microflora of aquatic environment for xenobiotics removal. Mangroves sediments contain 104-106cellsg^{-1} of aromatic-degraders and these indigenous communities have a considerable potential to degrade oil components. Pollutant from fresh water as well as contaminated tidal water accumulates in Mangroves wetlands considered as significant sinks for pollutants. The spilled and stranded oils containing large amounts of polycyclic aromatic hydrocarbons (PAHs) would penetrate and accumulate in mangrove sediments (Guo *et al.*, 2005).

Four isolates show rich and three were moderate upto 800ppm anthracene. With increasing concentration further upto 1200ppm only two isolates were best on anthracene. It is reported earlier that the isolation on anthracene in growth media during enrichment process were more adapted to degrade other PAHs. Adaptation processes, which occur as a result of an increase in the hydrocarbon-oxidising potential of the microbial community, allow the development of microbial populations with the ability to degrade PAHs (Macleod and Semple, 2006; Ye *et al.*, 2011). Adaptation was carried out through changing PAHs concentration in the growth media. The bacterial isolates subjected to different concentration showed concentration dependent growth pattern. Adaptation of microbes is an important step prior to any degradation process as exposure to high quantity of contaminants can be lethal to bacterial system. With increasing concentration enhanced growth rate was observed on the media plates is an indication of adaptation of the isolates. Furthermore, the number of oil-degraders could be increased by oil addition is an indication that microbes can adapt themselves by rapid multiplication when comes in contact with contaminants in their vicinity. As previously reported, these adaptation processes, even though not fully understood, are considered to be controlled by a

number of contaminant and soil factors including the concentration of contaminant, interactions with the indigenous microbial populations and length of contaminated soil contact time. In addition, microbial populations may undergo bioavailability-induced adaptations, which serve to aid contaminated microbe interactions and thus increase contaminant bio-accessibility (Kastner *et al.*, 1998; Johnsen *et al.*, 2005).

The screening media was changed from simplest nutrient agar to PNRG and then PNR with selected PAHs as the only carbon source showed that the growth of microbes is not affected by media. It is clear from this study, the enhanced growth rate was observed even after changing media from that containing an additional growth media (glucose) to one containing mineral PNR media with only PAHs as carbon source. The mechanism of adaptation depends not only, on the interaction of microbes with soil but with the amount of bioavailable contaminants as well. Previous exposure of indigenous microbes to PAHs can influence degradation capabilities, as suggested by a number of studies (Kastner *et al.*, 1998; Sartoros *et al.*, 2005; Puglisi *et al.*, 2007; Ye *et al.*, 2011). Naphthalene and phenanthrene biodegradation in soil always remain the main focus of research in terms of contaminants removal. The presence of one contaminant effect the accessibility of the other in a specified area as contaminants always exists in groups (Macleod and Semple 2006; Khan *et al.*, 2006; Kumar *et al.*, 2010).

PAHs degradation on solid media cannot be carried out completely. It was observed during this study that the isolates able to assimilate 1200 and 1000ppm anthracene and pyrene respectively. In order to quantify the degradation ability of these microbes were screening in liquid media was carried out and it was observed that they these PAHs compounds as their sole carbon and energy source. When these isolates were subjected, to the three ringed and four ringed PAHs were found to lose the ability to grow even (Alias *et al.*, 2011). Our isolate W_1, W_8 and W_{11} showed best growth on pyrene and phenanthrene individually but, loss the ability to grow on PAHs in mixture. Unlike previously reported work on *Sphingobium* sp. was found to be capable of utilizing naphthalene and biphenyl individually carried out complete degradation of naphthalene and biphenyl within 72 and 96hours respectively. However, growth was not observed on the possible metabolites of naphthalene and phenanthrene like 3-hydroxy-2-naphthoic acid, phthalic acid, protocatechuic acid, gentisic acid and catechols (Roy *et al.*, 2013).

During this study our isolate W_2 form pyrene-dihydrodiol (65.91%) and 1-methoxy-2, hydroxyanthracene (29.73%), after 48hours of incubation. The latter is the possible metabolite of anthracene as previously reported (Neelofur *et al.*, 2014). Phenanthrene and naphthalene degradation was carried out completely removed from the media in 72hours. Detection of catechols (64.05%), phthalic acids (31.44%) and benzocaumarin (32.89%) can be the possible metabolites of two and three ring PAHs compound by bacteria. 1-hydroxy-2- naphthoic acid (63.37%) were formed after 96 and 120hours respectively indicates that degradation was carried out through phthalic acid pathway. A small increase in production of phthalic acid (32.03%) and catechol (63.77%) were detected on further 24hours incubation as reported earlier (Khan *et al.*, 2015). *Pseudomonas* from municipal was capable of degrading phenanthrene as sole sources of carbon and energy and the metabolic pathways involved in the degradation of phenanthrene is also reported previously. Production of 1-hydroxy-2-napthoic acid, 1, 2-di-hydroxyanthracene and pyrenequinone by our isolates W_2 are the characteristic products of the PAHs oxidation by *Bacillus* sp. These metabolites were also detected by *Pseudomonas* isolated from municipal wastes that was capable of degrading phenanthrene (Joner and Leyval, 2003).

After 168hour benzocaumarin (93.13%) and traces of pyrene metabolites were detected during the degradation of the PAHs mixture the compounds identified were pyrene dihydrodiol and methoxy-2-hydroxyanthracene.

This shows that the further incubation products benzocaumarin, salicylaldehyde and phathalic acid and catechols recovered may be produced from pyrene after 168hours. Production of benzocaumarin and traces of pyrene metabolites were detected in this study are already reported from a no of studies during co-metabolism of PAHs (Fu-min *et al.*, 1993; Herwijnen *et al.*, 2003; Garcia *et al.*, 2004; Rodrigo *et al.*, 2005; Mallick *et al.*, 2007; Neelofur *et al.*, 2014).

Conclusion

It can be concluded from the present study, that sewage waste water contains bacteria capable of PAHs. These isolate can be adapted on solid media for degradation of high molecular weight PAHs like pyrene. Bacterial isolates can degrade PAHs on solid media but, when studied for quantitative study in liquid mineral media were unable to utilize these contaminants. Isolates which cannot degrade certain PAHs compound individually can completely convert to phathalic or catechol during co-metabolism.

References

Alias S, Talib SA, Omar M, Hussain NH. 2011. "Degradation of 3-ring PAHs by *S. spiritovorum and C. urealyticum* Strain under Non- Indigenous Condition," in International Congress of Chemistry and Environment (ICCE 2011), Port Dockson Malaysia.
www.sites.google.com/site/suhaimiabdultalib/resume

Arulazhagan P, Vasudevan N. 2011. Biodegradation of polycyclic aromatic hydrocarbons by a halotolerant bacterial strain Ochrobactrum sp. VA1. Marine Pollution Bulletin **62,** 388–394.
www.ncbi.nlm.nih.gov/pubmed

Bamforth SM, Singleton I. 2005. Rev. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. Journal of Chemical Technology and Biotechnology **80,** 723–736.
DOI: 10.1002/jctb.1276

Bastiaens L, Springael D, Wattiau P, Harms H, Dewachter R, Verachtert H, Diels L. 2000. Isolation of Adherent Polycyclic Aromatic Hydrocarbon (PAH)-Degrading Bacteria Using PAH-Sorbing Carriers. Applied and Environmental Microbiology **66,** 1834–1843.
www.ncbi. nlm.nih.gov

Chaineau CH, Morel J, Dupont J, Bury E, Oudot J. 1999. Comparison of the fuel oil biodegradation potential of hydrocarbon-assimilating microorganisms isolated from a temperate agricultural soil. Science of the Total Environment **227,** 237–247.
www.science.base.gov/catal og/item

Chaineau CH, Rougeux G, Oudot YC. 2005. Effect of nutrient concentration on the biodegradation of crude oil and associated microbial population in the soil. Journal of Soil Biology and Biochemistry **37,** 1490–1497.
www.hindawi. com/journals/btri

Clayton L. 2005. Rugh, Endang Susilawati, Alexandra N. Kravchenko, John C. Thomas Biodegrader Metabolic Expansion during Polyaromatic Hydrocarbons Rhizoremediation. Plant and Soil Science **60,** 331-339.
www.scholars. opb.msu.edu

Dabestani R, lvanov IN. 1999. A Compilation of Physical, Spectroscopic and Photophysical Properties of Polycyclic Aromatic Hydrocarbons. Photochemistry and Photobiology **70,** 10-34.
DOI: 10.1111/j.1751-1097.1999.tb01945.x

Dastgheib SM, Amoozegar MA, Khajeh K, Shavandi M, Ventosa A. 2011. Biodegradation of polycyclic aromatic hydrocarbons by a halophilic microbial consortium. Applied Microbiology and Biotechnology **95,** 789-798.
DOI: 10.1007/s00253-011-3706-4

Ebrahimi M, Sarikhani MR, Fallah R. 2012. Assessment of biodegradation efficiency of some isolated bacteria from oil contaminated sites in solid and liquid media containing oil-compounds. International Research Journal of Applied and Basic Sciences **3,** 138-147.
www. irjabs.com

Fu MM, Bruce MA, Garys SN. 1993. NAH plasmid mediated catabolism of anthracene and phenanthrene to naphthoic acid. Applied Environmental Microbiology **59,** 1938-1942.
www. scholar. google.com/citations

Garbeth A, Krausse S, Gemende B, Muller RH. 2004. Search for microorganisms that degrade PAH's under alkaline conditions. Engineering and Life Sciences **4,** 4.
www.ncbi.nlm.nih.gov

Garcia MT, Mellado E, Ostos JC, Ventosa A. 2004. Halomonas organivorans sp. nov., a moderate halophile able to degrade aromatic compounds. International Journal of Systematic and Evolutionary Microbiology **54,** 1723–1728.
www.ncbi.nlm. nih.gov/pubmed

Guo CL, Zhou HW, Wong YS, Tam NFY. 2005. Isolation of PAH-degrading bacteria from mangrove sediments and their biodegradation potential. Marine Pollution Bulletin **51,** 1054–1061.
www.hdl.handle. net

Haritash AK, Kaushik CP. 2009. Review on Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs). Journal of Hazardous Material **169,** 1–15.
www.ncbi.nlm.nih.gov/pubmed

Hassan EE, Hafez EE, Hussain AA, Ali AG, Hanafy AAE. 2009. Isolation and identification of three-ring polyaromatic hydrocarbons (anthracene and phenanthrene) degrading bacteria. American Eurasian Journal of Agricultural & Environmental Sciences **5,** 31-38.
http://dx.doi.org/10.5539/ijc.v5n4p43

Hazen TC. 2010. Cometabolic Bioremediation. In Handbook of Hydrocarbon and Lipid Microbiology. Springer-Verlag Berlin Heidelberg p. 2505-2514.
www.escholarship.org/uc/item/7vp2861h

Herwijnen RD, Springael P, Slot H, Govers AJ, Parsons JR. 2003. Degradation of Anthracene by *Mycobacterium* sp. Strain LB501T Proceeds via a Novel Pathway, through *o*-Phthalic Acid. Applied and Environmental Microbiology **69,** 186-190.
www.ncbi.nlm.nih.gov

Huang L, Sturchio NC, Abrajano JT, Heraty LJ, Holt BD. 1999. Carbon and chlorine isotope fractionation of chlorinated aliphatic hydrocarbons by evaporation. Organic Geochemistry **30,** 777-785.
DOI: 10.1016/S0146-6380(99)00060-1

Janjua NZ, Kadir MM, Lutfi S, Tipre M, Sathiakumar N. 2013. Tasman Spirit Oil Spill in Pakistan– Research Response and Lessons Learned Dr PH. American Journal of Indian Medicine **56,** 124–131.
DOI:10.1002/ajim.22039

Johnsen AR, Wick LY, Harms, H. 2005. Principles of microbial PAH-degradation in soil. Environmental Pollution **133,** 71-84.
DOI: 10.1016/j.envpol.2004.04.015

Joner EJ, Leyval C. 2003. Rhizospheric gradient of Polycyclic Aromatic Hydrocarbon (PAH) Dissipation in two industrial soils and impact of Arbuscular Mycorrhiza. Environmental Science and Technology **37,** 2371-2375.
DOI: 10.1021/es020196y

Kastner M, Breuer JM, Mahro B. 1998. Impact of inoculation protocols, salinity and pH on degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil. Applied and Environmental Microbiology **64,** 359-62.
www.ncbi.nlm. nih.gov

Khan K, Naeem M, Arshed MJ, Asif M. 2006. Extraction and characterization of oil degrading bacteria. Journal of Applied Sciences **6,** 2302-2306.
DOI: 10.3923/js.2006/2303.2306

Khan SA, Hamayun M, Bibi N, Sherwani SK. 2015. Isolation, Screening and Co-metabolism of Polycyclic Aromatic Hydrocarbons by Soil Bacteria. American Eurasian Journal of Agricultural & Environmental Sciences **15,** 800-812.
DOI: 10.5829/idosi.aejaes.2015.15.5.93218

Khurshid R, Sheikh MA, Iqbal S. 2008. Health of people working/living in the vicinity of an oil-polluted beach near Karachi, Pakistan. East Mediterrian Health Journal **14,** 179–182.
www.nap.edu/read/12949/chapter/10

Kiyohara H, Nagao K, Yana K. 1982. Rapid Screen for Bacteria Degrading Water-Insoluble, Solid Hydrocarbons on Agar Plates. Applied and Environmental Microbiology **43,** 454-457. www.ncbi.nlm.nih.gov

Kobayashi H, Ritmann BE. 1982. Microbial removal of hazardous organic compounds. Environmental Science and Technology **16,** 170A–183A.
www.books.google.com.pk/books

Kumar A, Munjal A, Khan A, Singh RN. 2012. Mega plasmid mediated anthracene (PAH) degradation by novel strain "*Geobacillus stearothermophilus* AAP7919" isolated from oil contaminated soil. Journal of Pharmacy Research **5,** 381-384.
www.sgrrcollege.com/FacultyID/48/Faculty-of-Science.aspx

Kumar G, Singla R, Kumar R. 2010. Plasmid Associated Anthracene Degradation by *Pseudomonas* sp. Isolated from Filling Station Site. Nature and Science **8,** 89-94.
www.irjabs.com

Leungsakul T, Glenn R, Johnson, Wood TK. 2006. Protein Engineering of the 4-Methyl-5-Nitrocatechol Monooxygenase from *Burkholderia* sp. Strain DNT for Enhanced Degradation of Nitroaromatics. Applied and Environmental Microbiology **72,** 3933–3939.
DOI: 10.1128/AEM. 02966-05

Lily MK, Bahuguna A, Bhatt KK, Dangwal K. 2013. Degradation of Anthracene by a novel strain Brachybacterium paraconglomeratum BMIT637C (MTCC 9445). International Journal of Ecological Sciences **3,** 1242.
www.scholar.google.com/citations

Luke NU, Ugochukwu UE. 2012. The Role of pH in Nitrate-reducing Conditions in Degradation of Polycyclic Aromatic Hydrocarbons in Contaminated Soil Extract. Der Chemica Sinica **3,** 435-439. http://dx.doi.org/10.5539/ijc.v5n4p43

Macleod CJA, Semple KT. 2006. The influence of single and multiple applications of pyrene on the evolution of pyrene catabolism in soil. Environmental Pollution **139,** 455-460.
DOI: 10.1016/j.envpol. 2005.06.014

Maiti AK, Das S, Bhattacharyya N. 2012. Bioremediation of High Molecular Weight Polycyclic Aromatic Hydrocarbons by *Bacillus thuringiensis* Strain NA2. Journal of Science **72,** 4.
www. scialert.net/eboardlivedna.php

Mallick S, Chatterjee S, Dutta TK. 2007. A novel degradation pathway in the assimilation of phenanthrene by Staphylococcus sp. strain PN/Y via meta-cleavage of 2-hydroxy-1-naphthoic acid: formation of trans-2, 3-dioxo-5-(2-hydroxyphenyl)-pent-4-enoic acid. Microbiology **153,** 2104-2115.
DOI: 10.1099/mic.0.2006/004218-0

Menzie CA, Potochi BB, Santodonato J. 1992. Exposure to carcinogenic PAHs in the environment. Environtal Science and Technology **26,** 1278-1284.
DOI: 10.1021/es00031a002

Moreno A, Albertelli A, Fabiano M. 2009. Nematode response to metal, PAHs and organic enrichment in Mediterranean Sea. Marine Pollution Bulletin **58,** 1192-1201.
www.eurekamag.com/ research

Mukesh KDJ, Sindhu G, Prasanna KP, Balakumaran MD, Kalaichelvan PT. 2012. Effect of Triton X-100 on Degradation of Polycyclic Aromatic Hydrocarbons by *Pseudomonas* sp. PSS6 Isolated from Municipal Wastes Sediment. Advances in Applied Science Research **3,** 1383-1388.
www.pelagiaresearchlibrary.com

Neelofur M, Prasad SV, Mahesh M. 2014. Enhance the Biodegradation of Anthracene by Mutation from *Bacillus* species. BMR Biotechnology **140,** 1-19.
www.bmrjournals.com

Obayori OS, Salam LB. 2010. Degradation of polycyclic aromatic hydrocarbons: Role of plasmids. Scientific Research and Essays Academic Journals. **5,** 4093-4106.
www.academicjournals.org/SRE

Othman N, Hussain N, Talib SA. 2010. Degradation of polycyclic aromatic hydrocarbon by pure strain isolated from municipal sludge: Synergistic and cometabolism phenomenon. (ICENV) p. 86-90.
DOI: 10.1088/1757-899X/136/1/012054)

Park KS, Sims RC, Dupont R. 1990. Transformations of PAHs in soil systems. Journal of Environmental Engineering–ASCE **116,** 632-40. http://dx.doi.org/10.1061/(*ASCE*)

Powell CL, Nogaro G, Agrawal A. 2011. Aerobic Cometabolic Degradation of Trichloroethene by Methane and Ammonia Oxidizing Microorganisms Naturally Associated with *Carex comosa* Roots. Biodegradation **22,** 527-538.
www.researchgate.net/publication

Puglisi E, Cappa F, Fragolis G, Trevisan A, Del-Re, AAM. 2007. Bioavailability and degradation of phenanthrene in compost amended soils. Chemosphere **67,** 548-556.
www.ncbi.nlm. nih.gov/pubmed

Rodrigo J, Jacques S, Santos EC, Bento FM, Maria CR, Peralba PA, Selbach ELS, Sa FAOC. 2005. Anthracene biodegradation by Pseudomonas sp. isolated from a petrochemical sludge land farming site. International Biodeterioration and Biodegradation **56,** 143-150.
DOI: 10.1016/j.ibiod. 2005.06.005

Roy M, Khara P, Basu S, Dutta TK. 2013. Catabolic versatility of *Sphingomonas* sp. strain BNP capable of degrading structurally diverse aromatic compounds. Journal of Bioremediation and Biodegradation **4,** 1-6.
http://dx.doi.org/10.4172/ 2155-6199.1000173

Rui L, Young MK, Kenneth FR, Thomas KW. 2004. Metabolic pathway engineering to enhance aerobic degradation of chlorinated ethenes and to reduce their toxicity by cloning a novel glutathione-*S*-transferase, an evolved toluene *o*-monooxygenase, and *γ*-glutamylcysteinesynthetase. Environmental Microbiology **6,** 491-500.
www.idosi.org /aejaes/jaes

Sartoros C, Yerushalmi L, Beron P, Guiot SR. 2005. Effects of surfactant and temperature on biotransformation kinetics of anthracene and pyrene. Chemosphere **61,** 1042-1050.
DOI: 10.1016/j.chemos phere.2005.02.061

Semprini L, Dolan EM, Hopkins GD, Perry LMC. 2005. Development of Effective Aerobic Cometabolic Systems for the In Situ Transformation of Problematic Chlorinated Solvent Mixtures. Strategic Environmental Research and Development Program. FINAL REPORT: ER-1127.
www. idosi.org/aejaes/jaes

Shokrollahzadeh S, Golmohammad F, Shokouhi H. 2012. Study of Sphingopyxis Isolates in Degradation of Polycyclic Aromatic Hydrocarbons. Chemical Engineering Transaction **27,** 55-60.
www.innspub.net

Siddiqi HA, Ansari FA, Munshi AB. 2009Assessment of hydrocarbons concentration in marine fauna due to Tasman Spirit oil spill along the Clifton beach at Karachi coast. Environment Monitoring and Assessment **148,** 139–148.
DOI: 10.1002/ajim.22039

Simarro RN, Gonzalez L, Bautista F, Molina MC. 2013. Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by a wood-degrading consortium at low temperatures. FEMS Microbiology and Ecolology **83,** 438–449.
DOI: 10.1089/10665270050081478

Sipkema E, Marjin W, Koning JK, Ganzeveld DB, Janssen A, Beenackers ACM. 2000. NADH-Regulated Metabolic Model for Growth of *Methylosinus trichosporium* OB3b. Cometabolic Degradation of Trichloroethene and Optimization of Bioreactor System Performance. Biotechnology Progress **16,** 189-198.
www.idosi.org/aejaes/jaes

Smith PN, Cobb GP, Codding GC, Hoff D, Scott TM, Thomas RR, Kevin DR. 2007. Contaminant exposure in terrestrial vertebrates. Environmental Pollution **150,** 41-64.
DOI: 10.5829/idosi.aejaes. 2015.15.5.93218

Soojhawon I, Lokhande PD, Kodam KM, Gawai KR. 2005. Biotransformation of nitroaromatics and their effects on mixed function oxidase system. Enzyme and Microbial Technology **37,** 527–533.
DOI: 10.1016/j.enzmictec.2005.03.011

Supakaa N, Pinphanichakarna P, Pattaragulwanita K, Thaniyavarna S, Omorib T, Juntongjin K. 2001. Isolation and Characterization of a Phenanthrene-Degrading *Sphingomonas* sp. Strain P2 and Its Ability to Degrade Fluoranthene and Pyrene via Cometabolism. Science Asia **27,** 21-28.
www.scienceasia.org

Survery S, Ahmad S, Subhan SA, Ajaz M, Rasool SA. 2009. Hydrocarbon degrading bacteria from Pakistani soil: Isolation, identification, screening and genetical studies. Pakistan journal of Biological Sciences **9,** 1518-1522.
www.pbs. org PJB

Tam NFY, Guo CL, Yau WY, Wong YS. 2002. Preliminary study on biodegradation of phenanthrene by bacteria isolated from mangrove sediments in Hong Kong. Marine Pollution Bulletin **45,** 316–324.
http://dx.doi.org/10.1016/S0025-326X(02)00108-X

Ting WTE, Yuan SY, Wu SD, Chang BV. 2011. Biodegradation of phenanthrene and pyrene by Ganoderma lucidum. International Biodeterioration and Biodegradation **65,** 238-242.
www.books. google.com.pk/books

Waqas M, Khan S, Chao C, Shamshad I, Qamar Z, Khan K. 2014. Quantification of PAHs and health risk via ingestion of vegetable in Khyber Pakhtunkhwa Province, Pakistan. Science of Total Environment **497,** 448-458.
http://dx.doi.org/ 10.1016/j.scitotenv.2014.07.128

Ye JS, Yin H, Qianga J, Penga H, Qin MH, Zhang N, He BY. 2011. Biodegradation of anthracene by Aspergillus fumigates. Journal of Hazardous Material **185,** 174–181.
DOI: 10.1016/j.jhazmat.2010.09.015

Yuan SY, Wei SH, Chang BV. 2000. Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture. Chemosphere **41,** 1463-1468.
http://dx.doi.org/10.1016/S0045-6535(99)00522-6

Zhong Y, Luan T, Wang X, Lan C, Tam NFY. 2007. Influence of growth medium on cometabolic degradation of polycyclic aromatic hydrocarbons by *Sphingomonas* sp. strain PheB4. Applied Microbiology and Biotechnology **75,** 175–186.
DOI: 10.1007/s00253-006-0789-4