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NAPHTHALENE AND PHENANTHRENE DEGRADATION BY PHYLLOSPHERE BACTERIA FROM THE ORNAMENTAL PLANTS IN URBANIZED AND POLLUTED AREAS OF SRI LANKA

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ABSTRACT

Polyaromatic hydrocarbonic (PAH) air pollution through vehicular and industrial emission is a highly concerned great threat in the modern world. The ability of bacteria, highly abundant in the phyllosphere of polluted and urbanized areas to degrade these deposited PAH pollutants into non-toxic levels was assessed and analyzed the concentrations of deposited PAH pollutants in the phyllosphere. Leaf samples of four ornamental plants species (*Ixorachinensis, Evertamiadervaticata, Hibiscus rosa-sinensis* and *Amaranthus cruentus*) were randomly collected from five polluted and a less polluted area as the control to isolate the PAH degrading phyllosphere bacterial species. Then plate assays, colorimetric methods and HPLC analysis were used to determine the best PAH degrading bacteria and subsequently identified them up to species level using molecular techniques. HPLC results revealed leaf phyllospheres of the polluted areas had significantly higher phenanthrene and naphthalene concentrations compared to the control site and phyllosphere bacterial population of the polluted areas were significantly higher than that in the less polluted site. As per HPLC and colorimetric results, out of twenty PAH degraders seven bacterial strains (*Alcaligenes faecalis*, *Alcaligenes* sp.11SO, *Bacillus cereus*, *Bacillus methylotrophicus*, *Serratia marcescens, Alcaligenes* sp. BC and *Alcaligenes* sp. GC)showed significantly higher PAH degradation abilities. Data indicates that, *Alcaligenes feacalis* was the best naphthalene and phenanthrene degrader with 98% and 88 % degradation abilities respectively. Phenanthrene and naphthalene concentrations high, phyllosphere of polluted areas had significantly higher PAH degrading bacterial populations compared to the control site. Out of seven phenanthrene and naphthalene degrading bacteria, *Alcaligenes feacalis*is especially an efficient bioremediator, because of its multi PAH degrading ability. The promising results of the present investigation will broaden the perspective of practical application of the above bacterial strains at environmental sites where contamination is caused by PAHs especially, phenanthrene and naphthalene.

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Keywords: Phyllosphere, *Alcaligenes faecalis*, phenanthrene, air pollution and naphthalene

INTRODUCTION

Polyaromatic hydrocarbons (PAHs) are the major hazardous pollutants in the ambient air which have high carcinogenicity and genotoxicity to all living beings (Rengarajan et al, 2002). In the United States Soil Protection Agency's list of priority pollutants, phenanthrene and naphthalene take first and fourth places respectively (Keith & Telliard, 1979). Vehicular emission, oil refining processes and industrial processes are the major sources of PAH pollutants to the ambient air (Harvey, 1991) when they deposit on the ground level surfaces through the dry deposition and wet depositions(Wild and Jones, 1995). Among many surfaces, leaf phyllosphere is the main exposing surface for the deposition of PAH pollutants.

Phyllosphere which is dominated by leaves represents the largest biosphere-atmosphere inter-phase on earth (Vorholt, 2012). Like other biological surfaces, the phyllosphere harbors a large number of diverse microorganisms, including bacteria, yeasts, oomycetes, fungi, and algae (Lindow and Leveau, 2002). The predominant microorganisms in the phyllosphere are bacteria and fungi (Lindow and Brandl, 2003). Phyllosphere of plants abundant in the highly polluted and urbanized areas are rich in both deposited PAH pollutants and microbes which can degrade PAH in significant levels (Karnchanasest and Satayavibul, 2005, Undugoda et al, 2016). Yutthammo et al, in 2010 revealed that out of many microbes isolated from ornamental plants in Chulalongkorn University area, which is located in the urban area of Bangkok, *Acinetobacter*, *Pseudomonas*, *Pseudoxanthomonas*, *Mycobacterium*, and uncultured bacterial species which were in the range of 1-10 % have a significantly high ability in PAH degradation over other organisms. Genera of *Pseudomonas*, *Microbacterium*, *Rhizobium,* and *Deinococcus* inhabiting the phyllosphere of *Ixora* spp. in the roadsides of Thailand were identified as the most efficient phenanthrene degraders (Waight et al, 2007). As indicated by Ilker et al, 2000 the bacterial strain *Rhodococcus* sp. isolated from a fuel oil-contaminated soil in Idaho, USA, was identified as a good naphthalene degrader.

Although some of the phyllosphere bacterial species were identified as efficient PAH degraders, the published literature in this area is very limited especially in Sri Lanka. Therefore, the present investigation was carried out with an attempt to identify naphthalene and phenanthrene degrading phyllosphere bacteria from highly polluted areas in Sri Lanka based on the hypothesis that most of the frequently occurring phyllosphere bacteria of ornamental plants of highly polluted and urbanized areas are very efficient in degrading phenanthrene and naphthalene. Thus, they have high surviving ability under harsh conditions and due to their particular abilities to degrade PAHs, they will be significantly useful in providing suitable criteria for remediating deposited PAHs from environments where contamination is caused by PAHs.

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MATERIALS AND METHODS

Leaf sample collection

Several leaf samples were randomly collected from the ornamental plants, *Ixorachinensis*, *Ervatamia divaricata*, *Hibiscus rosa-sinensis* and *Amaranthus cruentus* which are highly abundant in roadsides of five urbanized and industrialized areas, Colombo Fort, Orugodawattha, Maradana, Panchikawattha and Sapugaskanda in Sri Lanka. These sites have been believed as highly contaminated with PAHs emitted from vehicles and oil refinery processes. Leaf samples of same ornamental plants were also collected from the control site, Meemure, a remote rural area in Sri Lanka which is exposed to very low levels of pollutants compared with the sites in urban areas.

Isolation of phyllosphere bacteria

Fresh leaves sampled from each of the ornamental plants were used for phyllosphere bacterial isolations. Four grams of the fresh leaves (randomly selected) were placed in a flask containing 100 ml of potassium phosphate buffer (pH 7.0) and shaken at 180 rpm for 1 hr. A dilution series of 10^{-3}-10^{-9} was prepared using the leaf wash. One hundred µl aliquots of each dilution was then introduced to series of petri dishes containing mineral salt medium and were spread thoroughly in 50 replications. After a five day incubation period at room temperature 25 ^{o}C, bacterial colonies in the petri dishes were counted and each colony was subsequently streaked on Bacto Bushnell Haas (BBH) medium (pH 7.0 at 25°C) which contains $MgSO_4$ 0.2 g/L, $CaCl_2$ 0.02 g/L, $KHPO_4$ 1.0 g/L, K_2HPO_4 1.0 g/L, NH_4NO_3 1.0 g/L and $FeCl_3$ 0.05 g/L to which 100 ppm of PAHs (naphthalene and phenanthrene) were separately incorporated. The growth ability of streaked each bacterial species was investigated in the presence of PAHs. Following this, the number of PAH degrading bacterial colonies in each leaf wash was determined and PAH degrading bacterial population, in terms of colony forming unit (CFU/g) were calculated using the formula mentioned in Sharma et al, 2001 and Undugoda et al, 2016.

$$\text{CFU/g} = \frac{AxBxC}{D}$$

A: Average number of bacterial colonies; B: Final dilution; C: Volume of water used for suspension of sample; D: Weight of leaf sample.

The frequency of occurrence of PAH degrading bacteria on the leaf phyllosphere was then calculated using the following formula indicated by Desai et al, 1993.

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$$\text{Frequency of occurrence} = \frac{\text{Average population of individual PAH degrading bacterial species in all replicates}}{\text{Average of total bacterial population in all replicates}} \times 100$$

Based on the values obtained for the frequency of occurrence of PAH degrading bacteria were then categorized into the categories of dominant (81-100%), common (61-80%), frequent (41-60%) and occasional (40%).

Screening of phenanthrene and naphthalene utilization by phyllosphere bacterial strains

A Bacto Bushnell Haas (BBH) agar plate was divided into 25 squares and then each square is inoculated with the same bacterial strain. After three days of incubation period, each colony was transferred to the same type of 100 ppm PAH (phenanthrene and naphthalene separately) supplemented BBH agar plate which was divided into 25 squares as done for initial agar plate. The number of squares with colony growth out of 25 was then counted after seven day-incubation. Then the selected phenanthrene and naphthalene utilizers were further tested quantitatively for their PAH degradation using colorimetric and HPLC methods.

Quantification of phenanthrene and naphthalene degradation by phyllosphere bacterial species using colorimetric method

As indicated in Okafor et al, 2009 and Undugoda et al, 2016 the PAH degradation potential of isolated phyllosphere bacterial species was detected using a modified colorimetric method. "Two agar plugs (1 cm^2 each) of a pure growth of each isolate, were inoculated into Bacto Bushnell – Haas broth (50 ml/250 Erlenmeyer flask) with sterile PAH (100 ppm), redox indicator, methylene blue (2% v/v) and Tween 80 (0.1% v/v). The control flask was set up similarly without any bacterial species. Incubation of flasks containing bacteria was done at room temperature (28-30°C) with constant shaking at 180 rev/min for 7 days. After incubation, each culture was filtered to separate biomass and other residues followed by centrifugation at 8000 rpm for 15 minutes. Subsequently, absorbance of the supernatant was measured at 609 nm (for methylene blue) by using a UV-Vis spectrophotometer and the % of degradation of each PAH by each bacterial species was calculated separately using the absorbance values" (Undugoda et al, 2016).

Analysis of PAH degradation ability of the phyllosphere bacterial strains using HPLC

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The bacterial strains used in the colorimetric method were subjected to quantitative analysis of their phenanthrene and naphthalene degradation ability by using the HPLC method. As for the colorimetric method two agar plugs (1 cm^2 each) of each isolate were inoculated into BBH broth (50 ml) containing 100 ppm of PAHs (phenanthrene and naphthalene separately) and kept for a 7 day incubation period at room temperature. The residual PAHs in the broth were then extracted into acetone and hexane solvents using a chromatographic column (250 mm long, 4.6 mm diameter) after clean up procedure was carried out. The extracts were analyzed by Agilent 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with an Agilent 1200 diode array detector. Each sample (20 µl) was injected into A ZORBAX ECLIPSE Plus C18 column (Agilent Technologies, USA) (4.6 × 100 mm × 3.5 µm particle size). The mobile phase for PAHs was acetonitrile-water mixture (75:25) at a flow rate of 1.0 ml min^{-1}. Using 1, 10, 20, 40, 60, 80 and 100 ppm of PAHs phenanthrene and naphthalene a standard curve was plotted separately for phenanthrene and naphthalene. PAH concentrations in the samples were calculated against the standard curves and % of PAH degradation was calculated by the following formula

PAH degradation%=100 × [(Mi-Ms)/Mi]
Ms- The concentration of PAH in each treatment
Mi-The initial PAH concentration present in the sample (Tang et al, 2011).

HPLC analysis of the deposited phenanthrene and naphthalene on the leaf phyllosphere

Ten grams of randomly sampled fresh ornamental leaves were immersed in 100 ml of hexane: acetone (1:1) and subsequently they were shaken at 180 rpm for 1 hr. The filtered leaf wash was then evaporated using a rotary evaporator. Impurities of the sample were removed by passing through the chromatographic column (250 mm long, 4.6 mm diameter) which was packed with totally porous spherical C-18 material (packed size, 5 µm). Phenanthrene and naphthalene composition in the extract were analyzed using an Agilent 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with an Agilent 1200 Diode array detector. A ZORBAX ECLIPSE Plus C18 column (Agilent Technologies, USA) (4.6 × 100 mm × 3.5 µm particle size) maintained at room temperature was used for this purpose. Acetonitrile-water mixture (75:25) was used as a mobile phase for PAHs at a flow rate of 1.0 ml min^{-1}.

Identification of the best PAH degrading bacterial strains

Morphological and biochemical tests were carried out to identify the bacterial strains upto genus level. Biochemical tests were done according to the methods given in the Bergey's manual of determinative bacteriology (Bergey and Breed, 1994).

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PAH degrading phyllosphere bacterial strains were identified up to species level by determination of the nucleotide sequence of 16S rDNA region of rDNA operon. 16S rDNA region of the PAH degrading phyllosphere bacteria was amplified by PCR with primer pair 27F (5'- AGA GTT TGA TCM TGG CTC AG -3') and 800R (5'- GAC TAC CAG GGT ATC TAA TC -3') (White et al, 1990).

The PCR mixtures (40 µl) contained 5 µl of the genomic DNA sample, 10 × PCR buffer containing 750 mM Tris-HCl (pH 8.8 at 25°C), 200 mM (NH4) 2 SO4, 0.1% Tween 20; 2.5 mM MgCl2; 0.16 mM dNTP Mix; 20 pmol of forward and reverse primers and 0.75 U Taq DNA polymerase (MBI, Fermentas, Lithuania). PCR amplification was performed using a thermal cycler (Eppendorf Mastercycler 5330) programmed as follows: 5 min of denaturation at 94°C; followed by 40 cycles of 94°C for 30 seconds, 1 min of annealing at 52°C for primer pair 27F (5'- AGA GTT TGA TCM TGG CTC AG -3') and 800R (5'- GAC TAC CAG GGT ATC TAA TC -3') (Weisburg et al, 1991) and 1 min of extension at 72°C. The thermal cycles were terminated by a final extension for 5 minute at 72°C. Then PCR product (20 µl) was run in a 1.5% agarose gel and a gel piece with resulted band (600 bp) (White et al, 1990) was excised.

The purified DNA samples were used for direct sequence determination using sequencing primers with an Applied Biosystems automated sequencer (ABI3730XL) at Macrogen, Seoul, Korea. Almost complete 16S rDNA region sequences of strains designated as 11SO, 1NDR, TWP, NDP, PA4, GC and BC were obtained and compared with nucleotide sequences available in GenBank using BLASTN searches (Altschul et al, 1990).

Statistical analysis

The means of ten replicates of colorimetric assay, plate assays and HPLC analysis were analyzed using the SPSS 16.0 software to obtain the standard error (SE), least significant difference (LSD), ANOVA and regression analysis.

RESULTS

Leaf phyllosphere of the four ornamental plants, *Ixorachinensis*, *Ervatamia divaricata, Hibiscus rosa-sinensis* and *Amaranthus cruentus* collected from the five polluted sites showed higher bacterial diversities compared to the less polluted site, Meemure. Forty percent of the isolated phyllosphere bacterial population (which ranged from $3.1x10^3$ – $9.4x10^6$ CFU/g) were able to degrade both phenanthrene and naphthalene. Compared to the PAH degrading bacterial population of the five polluted sites, the less polluted site had significantly very low amount of PAH degrading bacteria (Figure.1). The highest PAH degrading bacterial diversity (9.61×10^6) was observed in the phyllosphere of *Ixora chinensis* collected from Colombo Fort. Similarly the

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second highest PAH degrading bacterial population was observed from the phyllosphere of *Ervatamia divaricate* collected from the roadsides of Colombo Fort.

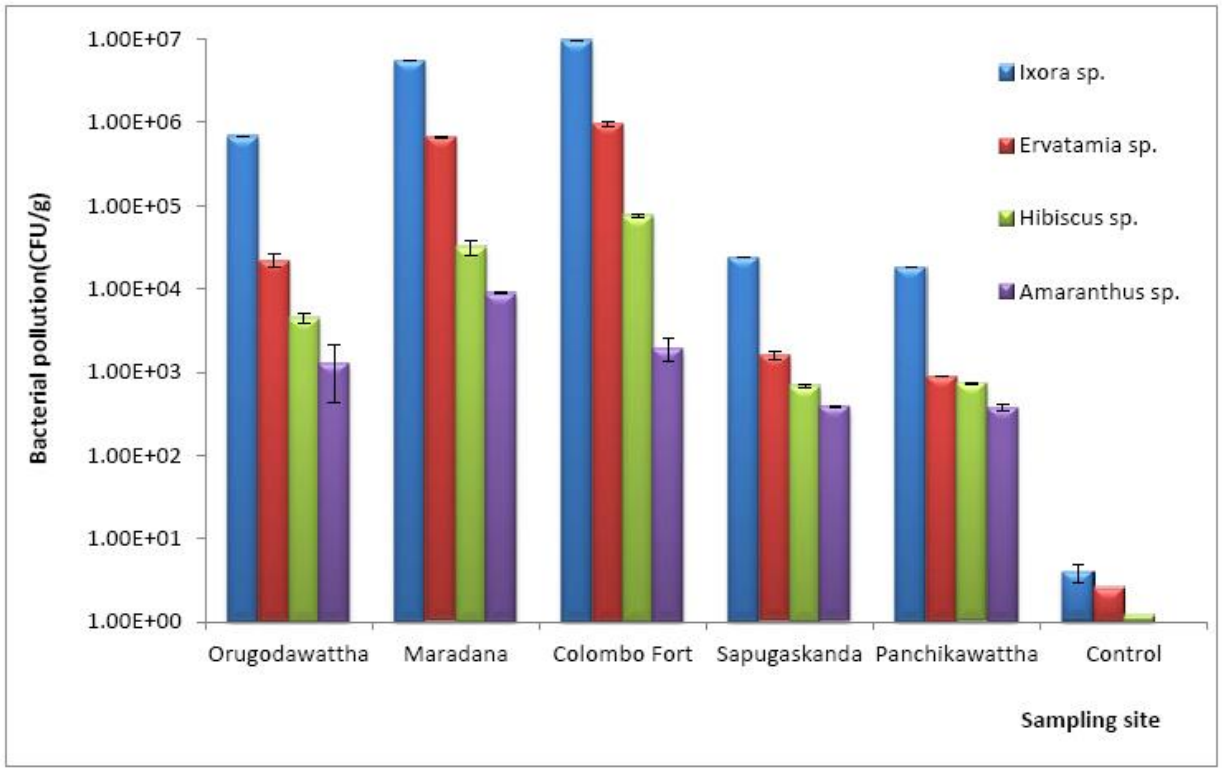

Figure. 1. AH degrading phyllosphere bacterial population (CFU/g) of polluted sites and control site (n=50)

Colombo Fort, Maradana and Orugodawattha showed significantly higher PAH degrading bacterial species compared to that of Sapugaskanda and Panchikawattha (Figure.1). AH degrading bacterial population of leaf samples collected from Sapugaskanda was higher than that of Panchikawattha.

Phenanthrene and naphthalene degradation of the phyllosphere bacterial strains

The phyllosphere of leaf samples was rich in different bacterial strains, which belong to different genera. Approximately 20bacterial strains were present on the phyllosphere of leaf samples from the five polluted areas. However, colony replica plate results of each bacterial strain revealed, out of these 20 strains, 11 strains were able to utilize AHs in the BBH medium. *Alcaligenes faecalis* and *Alcaligenes* sp. DN25 showed the highest AH utilization ability rather than other isolated bacterial strains (Table. 1).

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Table.1. Number of positive squares in the BBH medium supplemented with different AHs

Bacterial strain *	Phenanthrene	Naphthalene	Toluene	Xylene
Alcaligenes faecalis	21/25	24/25	11/25	6/25
Alcaligenes DN25	16/25	18/25	8/25	4/25
Bacillus sp.1	10/25	12/25	5/25	3/25
Bacillus sp.2	11/25	14/25	9/25	5/25
Psedomonas sp.1	9/25	11/25	9/25	6/25
Psedomonas sp.2	10/25	12/25	8/25	4/25
Klebsiella sp.	8/25	10/25	6/25	4/25
Serratia sp. 1	14/25	17/25	8/25	6/25
Serratia sp. 2	15/25	19/25	9/25	6/25
Acenatobacter sp.1	10/25	15/25	8/25	6/25
Acenatobacter sp.2	9/25	11/25	7/25	4/25

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These selected eleven bacterial species produced a color change in the BBH broth due to the reduction of the methylene blue indicator by the oxidation of polyaromatic hydrocarbon. The total color change (blue to colorless) supports the fact that the isolates are potential hydrocarbon oxidizers (Okafor et al, 2009) and this color change can be measured as an absorbance value using UV-Vis spectrophotometer.

The most efficient PAH degrading bacteria had significantly lower absorbance values after the incubation period compared to the control whereas poor PAH degraders showed significantly higher absorbance values compared to the control. Out of 11 PAH utilizing phyllosphere bacterial strains, 7 bacterial strains (belonging to 3 genera; *Alcaligenes, Bacillus, and Serratia*) were able to oxidize PAHs in the BBH broth (Figure. 2). As shown in the figure 2, bacterial strains *Alcaligenes faecalis*, *Alcaligenes* sp.11SO, *Bacillus cereus*, *Bacillus methylotrophicus*, *Serratia marcescens, Alcaligenes* sp. BC and *Alcaligenes* sp. GC were able to degrade naphthalene at 98 %, 92 %, 62%, 70 %, 55 %, 80 % and 51 % respectively. Bacterial strains *Alcaligenes faecalis*, *Alcaligenes* sp. 11SO, *Bacillus cereus*, *Bacillus methylotrophicus*, *Serratia marcescens, Alcaligenes* sp. BC and *Alcaligenes* sp.GC were able to degrade phenanthrene at 88 %, 84 %, 60 %, 45 %, 39 %, 51 % and 41 % respectively.

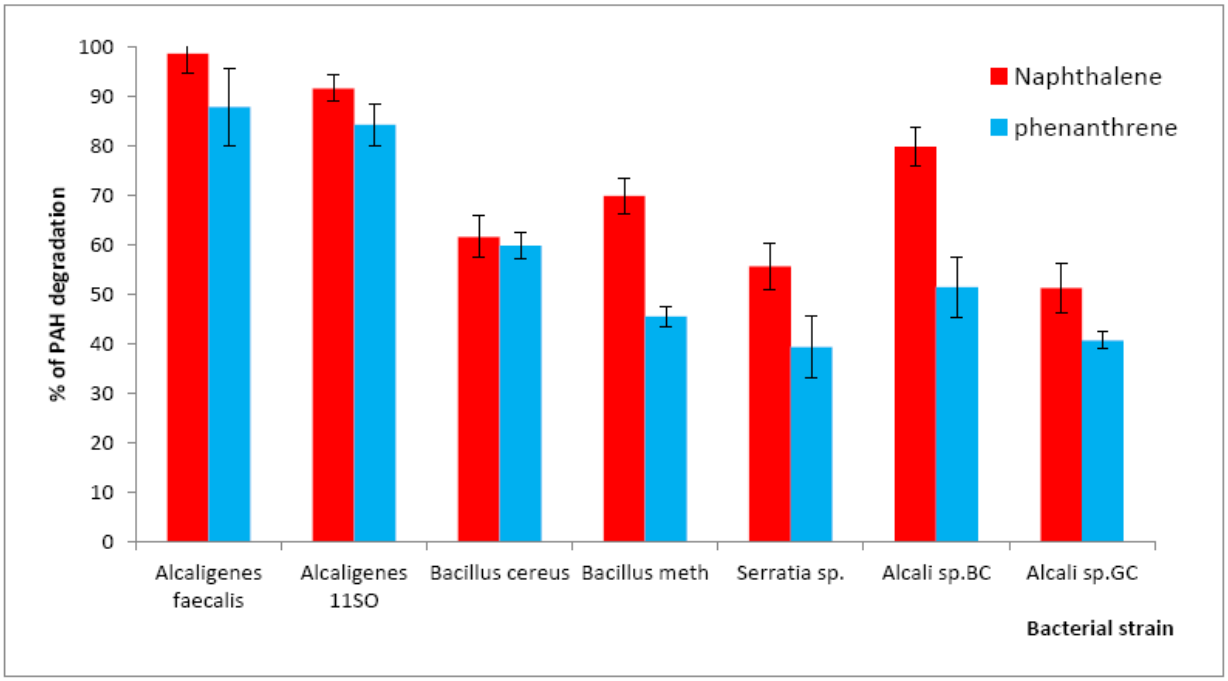

Figure 2. Percentage of polyaromatic hydrocarbon degradation by isolated bacterial strains, determined by colorimetric method (n=10).

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The HPLC results of selected phyllosphere bacterial strains revealed, *Alcaligenes faecalis* had the highest phenanthrene and naphthalene degradation ability, 89 % and 96 %, respectively (Figure. 3). Phenanthrene and naphthalene degradation ability of *Alcaligenes* sp.11SO and *Alcaligenes* sp. BC were significantly higher compared to the other phyllosphere PAH degrading bacterial strains.

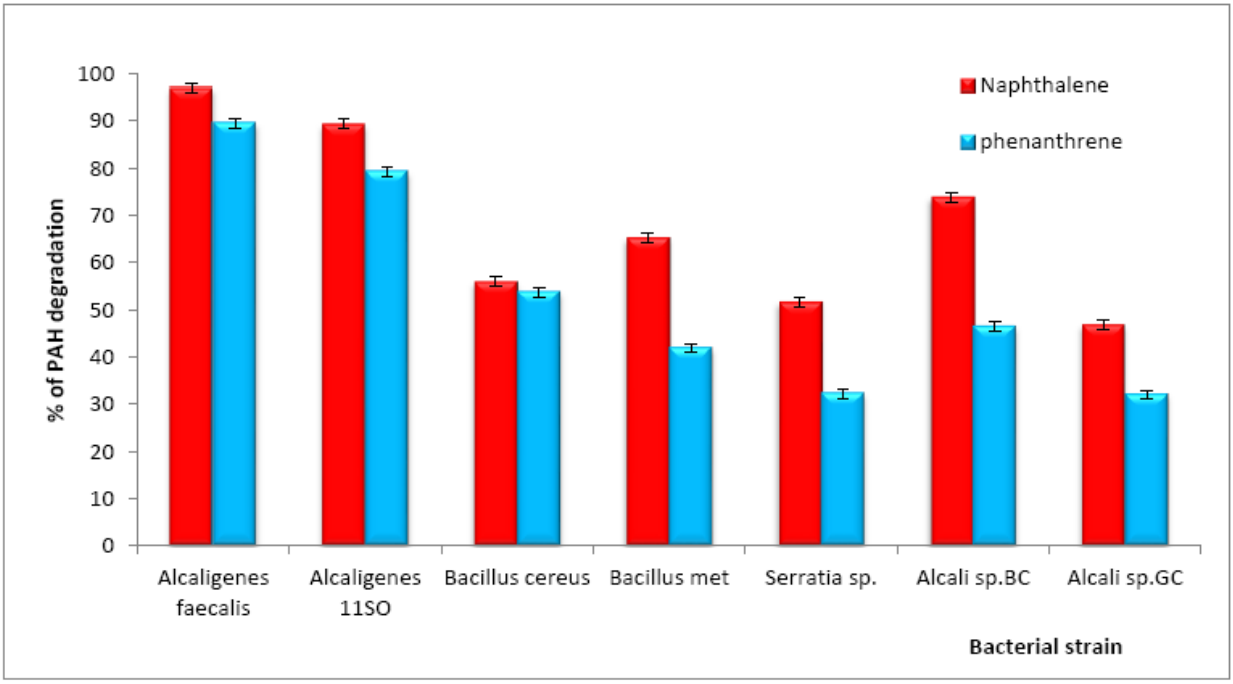

Figure 3. Percentage of polyaromatic hydrocarbon degradation by isolated bacterial strains, determined by HPLC method (n=10).

Frequency of occurrence of AH degrading phyllosphere bacteria

Following the results of frequency of occurrence, PAH degrading phyllosphere bacterial strains isolated from the five polluted sites and the control site were categorized as dominant (81-100 %), common (61-80 %), frequent (41-60 %) and occasional (0-40 %) as given in Sharma et al., 2001 (Table.2). The highly polluted sites dominated the most efficient PAH degrading strains. Although some of them are common to the less polluted site, they demonstrated significantly less abilities in degrading the same. The best PAH degrading bacterial strains, *Alcaligenes faecalis* and *Alcaligenes* sp. 11SO, were the dominant species in the phyllosphere of the ornamental plants collected from Colombo fort and Maradana (Table 1) but their occurrence is significantly less in Orugodawattha, Sapugaskanda and Panchikawattha. Other than that they were occasionaly isolated from the less polluted site. The present study reveals the capability of the

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above bacterial strains to degrade phenanthrene and naphthalene which sheds new light on the ways by which PAHs are degraded.

Table 2. Frequency of occurrence of the PAH degrading phyllosphere bacteria

Bacterial strains	Sample collection site					
	Maradana	Orugodawattha	Colombo Fort	Sapugaskanda	Panchikawattha	Control
Alcaligenes faecalis	Dominant	Common	Dominant	Common	Common	Occasional
Alcaligenes sp.11SO	Dominant	Common	Dominant	Common	Common	Occasional
Bacillus cereus	Common	Common	Dominant	Common	Frequent	Occasional
Bacillus methylotrophicus	Common	Frequent	Common	Frequent	Occasional	Occasional
Serratia marcescens	Common	Occasional	Dominant	Frequent	Occasional	Occasional
Alcaligenes sp.BC	Common	Occasional	Dominant	Frequent	Occasional	Occasional
Alcaligenes sp. GC	Frequent	Frequent	Common	Frequent	Occasional	Occasional

Identification of the best AH degrading phyllosphere bacteria

Gel electrophoresis of PCR products showed 600 bpamplicons representing entire 16S r RNA gene. Conserved region located in 16S r RNA gene of bacterial genome helps in identification of phylogeny of them by PCR method.

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Nucleotide sequences were compared with already existing DNA sequences in Genbank. The best AH degrading two strains shared 99 % 16S r RNA nucleotide sequence similarity with Genbank sequences. Comparison of the 16S r RNA nucleotide sequences of strains 11SO, BC and PA4 with nucleotide sequences of Genbank revealed that they were members of the genus *Alcaligenes*. Nucleotide sequence of 11SO was most closely related to *Alcaligenes faecalis* with 99% nucleotide sequence similarity. PA4 had also 99% nucleotide sequence similarity to *Alcaligenes* sp. DN25. Therefore, identity of the two best aromatic hydrocarbon degraders was established as *Alcaligenes* sp. DN25 (11SO) and *Alcaligenes feacalis* (PA4). BC had also 99% nucleotide sequence similarity to *Alcaligenes* sp. 2403 and GC had also 99% nucleotide sequence similarity to *Alcaligenes* sp. Nucleotide sequence of TNP strain was most closely related to *Bacillus methylotrophicus* in 99% nucleotide sequence similarity and NBD had 99% nucleotide sequence similarity to *Bacillus cereus.* The remaining bacterial strain was most closely related to *Serratia marcescens* 1NDR (99% sequence similarity). Accession numbers of isolates 11SO, PA, TNP, NBD, 1NDR, BC and GC submitted to the Genbank database were KT356809, KT356813, KT356814, KT356812, KT356808, KT356810 and KT356811 respectively.

DISCUSSION

The leaf samples of four plant species, *Ixorachinensis*, *Ervatamia dervaticata*, *Hibiscus rosa-sinensis* and *Amaranthus cruentus* collected from five polluted sites were rich in PAH degrading bacterial population compared to the less polluted control site. In fact, PAH degrading bacterial population ranged from $7.11 \times 10^3 - 8.2 \times 10^6$ CFU/g (Figure.1). According to the Waight et al, 2007 *Ixora* sp. collected from the polluted areas had the highest bacterial and fungal population. Similarly, out of four plant species collected from the five polluted sites *Ixorachinensis* showed the highest bacterial population. Waight et al, 2007 indicated high wax content on *Ixora* plant leaves leads to high PAH deposition in the phyllosphere. In fact, HPLC analysis data of *Ixorachinensis* leaf samples collected from Colombo Fort showed the highest phenanthrene (96 ng/g) and naphthalene (160 ng/g) (Undugoda et al, 2016) concentrations. According to the WHO records Colombo Fort is the highest PAH polluted site in Sri Lanka due to the high vehicular emission and industrial processes. Simultaneously, *Ixora* leaf samples collected from this area showed higher PAH degrading bacterial populations. Therefore, there was a proper correlation among the PAH pollutant level of the phyllosphere and bacterial population on it. Similarly the second highest PAH degrading bacterial population was observed from the phyllosphere of *Ervatamia divaricate* collected from the roadsides of Colombo Fort. As per results in Undugoda et al, 2016, HPLC analysis of the leaf phyllosphere of *Ervatamia divaricate* had significantly higher naphthalene and phenanthrene, concentrations compared to the surface of the leaves of other ornamental plants such as *Hibiscus rosa-sinensis* and *Amaranthus cruentus*.

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The PAH degrading phyllosphere bacterial consortium was higher in samples collected from Sapugaskanda and Orugodawattha, compared to that of the Panchikawattha area. However, Sapugaskanda is an area located out of the Colombo city, but the PAH concentration of leaf samples obtained from that site, was as high as that of the samples from Orugodawattha (Undugoda et al, 2016). The reasons for this are, the current location of the oil refinery, being an industrial zone and the prevalence of high vehicular traffic in the area, which are accounted for the significant increase in the PAH levels in the ambient air. Simultaneously, phenanthrene and naphthalene degrading phyllosphere bacterial population of leaf samples collected from Sapugaskanda was as high as Orugodawattha. All in all, there is a significant correlation between PAH degrading bacterial consortium in the leaf phyllosphere and the PAH concentration of the phyllosphere. The results of Chung, 2001, also revealed, a correlation of the accumulation of PAHs on plant leaves with the atmospheric PAH concentrations. Hence the presence of PAH degrading phyllosphere bacterial populations in significantly higher diversities can be considered as a proper bioindicator for the indirect measurements of PAH levels in the ambient air of urban areas.

As per the colorimetric and HPLC results, most of the phenanthrene and naphthalene degraders isolated from the leaf samples belong to the genus *Alcaligenes* and they were highly efficient in degrading phenanthrene as well as naphthalene. Findings of Kiyohara et al, 1982, bacterial strain *Alcaligenes* sp. AFK2 isolated from contaminated soil samples in Tokyo was able to degrade phenanthrene, but the strain was unable to degrade naphthalene. As indicated by Waight et al, 2007 most of the phyllosphere PAH degrading bacterial strains isolated from the ornamental plant leaves at roadsides of Bangkok belong to genus the *Pseudomonas* and they were highly efficient in degrading naphthalene and phenanthrene. In fact the results of Sun et al, 2014 revealed, an endophytic bacterium *Pseudomonas* sp. Ph6 which was isolated from clover (*Trifolium pratense* L.) grown in a PAH-contaminated site in USA had significantly higher phenanthrene degradation ability in contrast to the findings of the present study where most of the *Pseudomonas* sp. were unable to degrade phenanthrene efficiently. In agreement with the observation of the present study *Alcaligenes feacalis* isolated from the leaf samples of polluted sites showed the highest phenanthrene and naphthalene abilities.

Out of the several aspects tested it was confirmed that the best PAH degrading phyllosphere bacterial strain, *Alcaligenes faecalis* which dominates the phyllospheres of highly polluted sites could degrade both phenanthrene and naphthalene compared to other isolated polyaromatic hydrocarbon degrading bacterial species, during this research.

In summary the present investigation can be considered as the first experiment conducted in Sri Lanka which provides evidence for the biodegradation of PAHs (phenanthrene and naphthalene)

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by the phyllosphere bacterial species. This research data opens several avenues for further experiments in the relevant field and also indicates the possibility of using the efficient PAH degraders in remediating the polyaromatic hydrocarbon pollutants from environmental sites where high contamination prevails.

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CONFLICT OF INTEREST

No conflict of interest by authors of this manuscript.

REFERENCES

Altschul S.F, Gish W, Miller W, Myers E.W and Lipman D.J (1990) Basic local alignment search tool. *The Journal of Molecular biology*. vol. 215, pp. 403-410.

Bakker, M. I., Tolls, J., and Kolloffel, C. (2001) "Deposition of atmospheric semi volatile organic compounds to vegetation". *The Journal of Environmental Science*. vol. 24 pp. 286-298.

Bergey D.H and Breed R.S, (1957). Baltimore, Williams and Wilkins company, America.

Chung J. H, Lee S. H and Youn C. S, (2001), Cutaneous photodamage by polyaromatic hydrocarbons". *The journal of Dermatology*. vol. 137. pp. 1043-1051.

Desai A, Jitendra J, Desai D, Hanson K.G (1993) A rapid and simple screening technique for potential crude oil degrading microorganisms. *The Journal of Biotechnology Techniques* vol.10, pp.745-748.

Harvey R.G (1991) Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity. Cambridge University Press, Cambridge.

Ilker U.Z, DuanY.P and OgramA, (2000). Characterization of the naphthalene-degrading bacterium, *Rhodococcusopacus* M213. *The Journal of FEMS Microbiology Letters*. vol.185, pp. 231-238.

Karnchanasest B and Satayavibul A. (2005) Orange jasmine leaves as an indicator of atmospheric polycyclic aromatic hydrocarbons. *The Journal of Science Technology*. vol. 27, pp.877–888.

Keith L.H and Telliard W.A (1979) Priority pollutants. A perspective view. *The Journal of Environmental Science Technology*. vol.13, pp.416–423.

KiyoharaH, NagaoK, Kouno,K and YanoK (1982). Phenanthrene-degrading phenotype of *Alcaligenesfaecalis* AFK2. *The Journal of Environmental Microbiology*. vol.43. pp.458-461.

Lindow S.E, Brandl M.T(2003), Microbiology of the phyllosphere. *The Journal of Applied Environmental Microbiology*. vol.69(4), pp.1875–1883.

Lindow, S. E. and Leveau, J. H. J. (2002) "Phyllosphere microbiology review". *The Journal of Applied Environmental Microbiology*.vol.13, pp. 238–243.

Okafor G.U, Tasie F and Okafor M.F,(2009). Hydrocarbon Degradation Potentials of Indigenous Fungal Isolates from Petroleum Contaminated Soils. *The Journal of Physical Nature Science*, vol. 3(1): pp. 1-6.

Rengarajan T.S, Rajendran P, Natarajan N, Boopathy L, Rajendran P and Nishigaki I (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *The Journal of Asian Pacific Journal Tropical Bioremediation.* vol. 5(3): pp.182-189.

Sharma V.V, Hyde K.D and Vittal, B. P. R (2001). "Frequency of occurrence of mangrove fungi from the east coast of India". *The Journal of Hydrobiologia*. vol. 455: pp. 41-53.

Sun K, Liu J, Gao Y, Jin L, Gu Y and Wang W, (2014). Isolation, plant colonization potential, and phenanthrene degradation performance of the endophytic bacterium *Pseudomonas* sp. Ph6-gfp. *The Journal of Scientific Reports*. vol.4, pp. 5462-5471.

Tang Y.B, Yang X, Chen F.Y, Jiang R.L, Wang X.G (2011) Screening, identification and degrading gene assignment of a chrysene-degrading strain. *The Journal of African Journal of Biotechnology*. vol.10. pp. 6549-6557.

Undugoda L J S, Kannangara S and Sirisena D M (2016), Aromatic hydrocarbon degrading fungi inhabiting the phyllosphere of ornamental plants on roadsides of urban areas in Sri Lanka**.** *The International Journal of Biodegradation and Bioremediation*. vol.7: pp.328. doi: 10.4172/2155-6199.1000328.

Vorholt J.A (2012) Microbial life in the phyllosphere. *The Journal of Nature Review Microbiology*. vol. 10, pp. 828-840.

ISSN: 2455-6939

Volume:02, Issue:05

Waight K, Pinyakong O and Luepromchai E (2007).Degradation of phenanthrene on plant leaves by phyllosphere bacteria. *The Journal of General Applied Microbiology*. vol.53. pp.265-272.

Weisburg W.G, Barns MS, Pelletier A.D and Lane J.D. (1991). 16S ribosomal DNA amplification for phylogenetic study. *The Journal of Bacteriology*. Vol.173, pp.697- 703.

White T.J, Bruns T, Lee S, Taylor J and Innis A,(1990) PCR Protocols. Academic Press, San Diego, USA. pp: 315-322.

Wild, S. and Jones, K.C. (1995) Polynuclear aromatic hydrocarbons in the UK: a preliminary source inventory and budget. *The Journal of Environmental Pollution.* vol.88, pp.91-108

Yutthammo C, Thongthammachat N, Pinphanichakarn P and Luepromchai E (2010).Diversity and activity of PAH-degrading bacteria in the phyllosphere of ornamental plants. *The Journal of Microbial Ecology*. vol.59, pp.357-368.

