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Mangrove Bacterial Consortiums Erasing Benzo[a]pyrene Menace from Karankadu, Palk bay, Southeast coast of India

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental pollutants originating from in complete combustion or natural decay of organic matter. These contaminants, Marked by their hydrophobic nature, resist degradation, posing ecological and human health risks. Notably, benzo[a]pyrene (BaP), characterized by its intricate five-fused benzene rings is particularly concerning due to its mutagenic, carcinogenic, and teratogenic properties. To counter this issue, harnessing the biodegradative potential of microorganisms, given their cost-efficiency and inherent hydrocarbon metabolism ability, emerges as a promising solution. This study centers on the degradation of benzo[a]pyrene, a highly recalcitrant high-molecular-weight PAH, in marine environments. The research employs enriched bacterial isolates with in a consortium, focusing on saline conditions. Isolates extracted from enriched mangrove sediment show cased the ability to utilize benzo[a] pyrene as their sole carbon and energy source. The isolates, identified as Bacillus cereus PVS01(OQ954107), Achromobacter insolitus PVS02 (OQ95418), and Pseudomonas aeruginosa PVS03 (OQ954109), individually achieved degradation rates of 37%, 34%, and 24%, respectively, when exposed to an initial benzo[a]pyrene concentration of 20mg/L duringa10–day sea water incubation (with 28ppm NaCl). In contrast, a bacterial consortium managed to degrade 44% of an initial 50mg/L benzo[a]pyrene concentration over the same incubation period and saline conditions. Optimization of benzo[a]pyrene degradation was achieved by adjusting key factors, resulting in a 35°C temperature, neutral pH, and 1.5% NaCl concentration. This study underscores the potential of co-metabolism within a consortium as a viable strategy for benzo[a]pyrene biodegradation in seawater environments.

Keywords: *PAH degradation, Benzo[a]pyrene, Karankadu mangrove, Consortium, Biodegradation, Microbial degradation, HMW Polycyclic aromatic hydrocarbons.*

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) stand as persistent environmental pollutants, arising from both natural combustion processes and human activities. Their hazardous nature lies in their cytotoxic, mutagenic, and carcinogenic attributes. Consequently, the destiny of these substances within the environment, along with the restoration of PAH-contaminated zones, captures significant public attention. Swift and efficient eradication of PAH compounds from the environment assumes utmost importance to mitigate their adverse repercussions. Employing indigenous or native micro flora for bioremediation emerges as a compelling avenue, as it often proves more advantageous and effective compared to commercial inoculants, which could potentially be out competed by local microorganisms [1]. Various Populations of indigenous bacteria with the capacity to utilize and degrade pollutants such as PAHs may have existed within polluted sediments [2]. Researcher's successfully isolated bacteria from polluted sites that exhibited the ability to mineralize diverse PAHs [3].Further investigations revealed that exposing microorganisms to PAHs in sediments prior to their actual breakdown, and allowing them to acclimatize, led to enhanced degradation rates. The biodegradation potential of strains isolated from hydrocarbon-contaminated environments was found to be comparable to or even surpass that of bacteria from uncontaminated sediments. The marine ecosystem frequently faces contamination from PAHs, primarily

stemming from human activities such as accidental spillages and oil seepages from ships and petroleum industries. Additionally, incomplete combustion of hydrocarbon fuels, gasification, co liquefaction, and biomass combustion contribute to PAH pollution. PAHs represent a class of prevalent organic pollutants characterized by their configuration of two or more interconnected aromatic rings, forming linear, angular, or cluster arrangements. Due to their hydrophobic nature, these substances exhibit remarkable persistence, bioaccumulation potential, cytotoxicity, and carcinogenicity. Furthermore, their chemical last ability within the noteworthy [4]. These chemicals also possess an affinity for binding with particulate matter found in water, air, sediment, and soils. The properties mentioned above play a pivotal role in shaping the behavior of these chemicals within the environment. Their tendency to exhibit low availability for biological absorption is a crucial factor that contributes to their unique ecological impact. This characteristic means that these substances possess a distinct capability to resist being taken up by various organisms, there by setting the stage for a complex phenomenon known as bioaccumulation. This process involves the gradual build-up of these chemicals as they traverse through different levels of the food chain [5]. This intricate interplay of chemical properties and biological processes has caught the attention of global environmental organizations. The United Nations Environmental Programmed, recognizing the substantial ecological implications, has explicitly categorized sixteen specific PAHs as refractory organic pollutants. This designation underscores the challenge these Compounds present in terms of their persistence and potential to disrupt natural systems. The significance of this issue has also led the US Environmental Protection Agency (EPA) to accord these chemicals the status of priority pollutants, reflecting the urgent need for regulatory measures and targeted interventions to mitigate their adverse effects. Mangroves represent typical tropical ecosystems situated between terrestrial and marine environments. They are influenced by tidal patterns and create favorable conditions for animal nourishment, shelter, and reproduction [6]. Mangroves play a crucial role in transforming nutrients into organic matter, offering protection against erosion and extreme events, and delivering economic advantages to local communities [7,8]. However, these ecosystems are susceptible to human-induced pollution caused by PAHs originating from tidal water, river sources, and land-based origins. Research has indicated, elevated PAH concentrations in mangrove sediments, exceeding 10,000 ngg-1 dry weights [9]. Bacterial activity plays a crucial role in the establishment of substantial biomass. Within tropical mangroves, bacteria and fungi constitute are makeable 91% of the overall microbial biomass, while algae and protozoa contribute 7% and 2% correspondingly [10]. Due to their distinctive attributes such as high primary productivity, abundant detritus, ample organic carbon, and anoxic/reduced conditions, mangroves are a favored environment for the absorption and retention of PAHs originating from human activities. Researchers stated [11] that mangrove sediments harbor a considerable number of aromatic-degrading microorganisms (10^4–10^6 cells g^{-1} sediments), indicating their substantial capability to breakdown constituents of oil. Additionally, the introduction of oil can lead to an augmentation in the population of oil-degrading organisms. In interesting contend that a bacterial consortium derived from mangrove sediments exhibits are makeable capability for PAH degradation, show casing complete breakdown of both fluorine and phenanthrene within four weeks of cultivation [12]. These findings imply the presence of diverse PAH-degrading bacterial strains within mangrove sediments, each possessing significant PAH degradation potential. PAHs are categorized into two groups: low-molecular-weight (LMW) compounds with two or three rings, and high-molecular-weight (HMW) compounds with four or more rings, including pyrene (four rings) and BaP (five rings). Within the existing literature, numerous instances are detailed that outline the bacterial breakdown of LMW-PAHs, with relatively fewer studies delving into pyrene degradation[13,14]. Biodegradation of BaP has garnered Limited attention due to it resistance to microbial breakdown, stemming from the stability of the conjugated double bonds present in its fused rings [5]. BaP stands out as a significant member of the PAH family commonly encountered in the environment. Similar to other PAHs, BaP originates from petroleum derivatives, making its way into the surroundings through processes like oil extraction, transportation, storage, and even emissions from vehicular engines [15]. This compound is also present in coal tar and tobacco, originating from the incomplete combustion of organic materials within a temperature range of 300 to 600°C. This explains its occurrence in charred or grilled meats as well [16, 17]. Consequently, BaP has established itself as a pervasive contaminant, it slow solubility in water further contributing to its propensity for accumulation and subsequent toxicity in ecosystems, animals, and humans alike [15]. BaP is an acknowledged carcinogen, and its carcinogenic effect is attributed to its transformation into dihydroxy-epoxy tetrahydro-BaP, a process that leads to DNA binding (resulting in DNA adducts), introducing errors in DNA replication, and ultimately triggering Uncontrolled cell division and the development of cancer [18]. Furthermore, BaP exerts adverse effects on the development and functioning of the immune system, as well as on child fertility. These adverse impacts have been observed in animal models as well [4, 19]. As a result, the imperative to eliminate BaP from the

environment remains paramount. One promising approach is biodegradation, a mechanism reliant on the capacity of microorganisms to utilize pollutants as sources of carbon and energy. This strategy offers both cost-effectiveness and environmental friendliness for the removal of these pollutants from the environment. The primary objective of the present study is fourfold: (1). To ascertain the levels of PAHs in surface sediments of mangrove ecosystems; (2). To quantify the population of PAH-degrading bacteria, utilizing a model mixture of phenanthrene (a 3-ring PAH), pyrene, and fluoranthene (4-ring PAHs), benzo(a)pyrene (5-ring PAHs); (3). To explore the inherent biodegradation capabilities of microorganisms native to mangrove sediments; (4).To assess the potential of a bacterial consortium enriched from mangrove environments, to determine the BaP degradation ability of the enriched bacterial consortium.

MATERIAL AND METHODS

Study Area

The study area, Karankadu mangrove swamp (Latitude 9°36'N and Longitude 78°83'E), is situated along the southeast coast of India within the Palk Bay region (Latitude 9°55'-10°45'N and Longitude 78°58'-79°55'E). **Figure 1** depicted the study area's geographic map and sediment collection points. Within the Karankadu mangrove swamp, a diverse array of creatures including crabs, bivalves, fish, birds, and snakes have established their habitats. The entirety of the Karankadu village covers a coastal expanse of 400 hectares, with a flourishing stretch of mangroves encompassing approximately 102 hectares along the Kottakarai estuary banks. Notably, during high tide, the mangrove ecosystem receives saline water from the river, extending up to 5 kilometers away. The district receives 136.1mm of yearly rainfall from the south-west monsoon and 53.47.4 mm from the north-east monsoon [20, 21].

Sampling and PAH Analysis Methodology

Surface sediment samples were obtained from the upper 0–10 cm layer using as oil corer during low tide periods. These samples were carefully placed in pre-cleaned brown glass vials and kept frozen at -20°C for PAH analysis. Within 24 hours of collection, selected sediment samples were transferred to sterilized 100 mL plastic bottles for microbiological investigation. Three replicates were collected at each sampling point to ensure reliability. To determine PAH concentrations, a standard procedure following established guidelines was employed [22]. This entailed dichloromethane and methanol-based extraction, using m-terphenyl as an internal standard. The samples were then analysed using gas chromatography with flame ionization detection (GC-FID) on a Hewlett-Packard 5890 gas chromatograph. This meticulous approach facilitated the quantification of 16 distinct PAH compounds present in the sediment samples.

Microbial Assessment

To quantify PAH-degrading bacteria, a modified spray-plate technique was utilized. A mineral salt medium (MSM) composition containing specific components was prepared, and its pH was adjusted. Solid medium plates were incubated at 25°C overnight prior to inoculation. Bacterial cultures were prepared by mixing a 10 g sediment sample with 90 mL sterile water in a 250 mL conical flask. The mixture was agitated and allowed to settle before creating decimal dilutions for testing. For PAH-degrading bacteria assessment, stock PAH solutions were prepared at 0.5 mg. mL^{-1} in acetone [23]. Acetone a solution of model PAH compounds was sprayed onto plates, followed by addition of diluted sediment samples. Identification of PAH-degrading bacteria involved detecting clear-zone-forming colonies on mineral medium Plates coated with individual PAH compounds. After three weeks of incubation in darkness at 25°C, the presence of such colonies indicated the existence of PAH-degrading bacteria.

Enrichment and Isolation of PAH-Degrading Microbial Consortia

To cultivate and isolate PAH-degrading microbial consortia, a model mixture of PAH compounds (phenanthrene, fluoranthene, pyrene, and benzo(a)pyrene) was introduced at 50 mgL^{-1} concentration in to a 250 mL conical flask. This flask contained 90 mL of MSM along with a 10 g sediment sample derived from a composite of sediment collected from 7 different stations. Incubation at 25°C in darkness occurred for one week. After this initial incubation, 5 mL of the culture was transferred to another 250 mL conical flask containing 45 mL of MSM and the equivalent amount of PAHs, initiating a second round of enrichment. This collaborative process continued for two months within the laboratory. For bacterial colony isolation, the enriched consortium was streaked onto agar plates containing marine broth 2216E. Individual colonies were selected based on their distinct color and morphology, serving as preliminary markers for differentiation. Gene amplification was executed using PCR and the primers at 27F (position 8-27 of *Escherichia coli*, 5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (position 1510-1492 of *Escherichia coli*, 5'-GGCTACCTTGTTACGACTT-3'), as described in studies [24]. Subsequently, DNA sequences from cloned 16S rDNA fragments underwent a comparative analysis. BLASTN, a tool accessible at the National Center for Biotechnology Information (NCBI) via http://www.ncbi.nlm.nih.gov/BLAST/ facilitated this comparison.

BaP Degradation by Individual and Consortia Isolates

To assess BaP degradation, experiments were carried out in 250 mL flasks containing 49 mL of salt water supplemented with BaP as the sole carbon and energy source. Individual isolates were exposed to BaP doses of 20 mg/L, while consortiums were subjected to a higher dose of 50 mg/L. These BaP concentrations were dissolved in acetone. For the biodegradation trials, 1.0 mL of the enriched bacterial community at OD 600 0.1 to 0.2 was added to each medium. The flasks were covered with aluminum foil and placed on a rotary shaker at 150rpm in the absence of light. Throughout the incubation, samples of the culture media were periodically extracted at various time points to assess BaP levels. Duplicate flasks were setup for each treatment to determine residual BaP content. These samples were stored at 20°C until analysis. Gas chromatography (GC) combined with Flame Ionization Detection (FID) was employed for precise quantification of BaP concentrations, offering a robust method for evaluating the extent of BaP degradation [25].

BaP Analysis Methodology

To analyse residual BaP, a specific procedure was employed. Residual BaP in the sample underwent extraction using ethyl acetate (v/v) through a two-step process. The cultured medium was vigorously shaken for 10 minutes in a separate funnel, followed by settling to form two distinct phases. Organic solvents were combined and treated with a hydrous sodium sulfate for drying. The organic extract was then evaporated using a rotary evaporator, reducing the volume to 2 mL. This condensed extract was subjected to analysis using an Agilent technologies-7890 AGC-FID instrument. The analysis utilized an HP-5MS fused silica capillary column with nitrogen as the carrier gas. Column dimensions were 30 m length, 0.25 mm internal diameter, and 0.25 m film thickness. The gas chromatograph was configured with the injector operating in split less mode, and automated samplers introduced 1µL aliquots of the sample. These steps allowed precise quantification and characterization of BaP within the samples. The oven temperature was programmed following a specific thermal profile: starting at 60°C for 2 minutes, linearly increasing to 120°C at 10°C per minute, and further increasing to 300°C at a rate of 3°C per minute. The final temperature, 300°C, was maintained for 10 minutes. Detector and injector temperatures were set at 300°C and 280°C respectively, ensuring optimal analysis performance and accuracy. BaP biodegradation extent was determined by calculating the percentage of peak area. This method enabled quantification of BaP degradation achieved in the samples, offering valuable insights into the biodegradation process [25].

BaP Degradation Calculation and Kinetics Analysis

The BaP degradation percentage (ER) was calculated using the formula

$$\text{BaP removal efficiency (ER \%)} = (Ci - Cr)/Ci \times 100$$

Here, ER (%) denotes the removal efficiency percentage, Ci represents the initial BaP concentration, and Cr indicates the remaining BaP concentration. Biodegradation kinetics was modeled using a first-order kinetic model [26].

$$Ct = Co \times e \text{ ^}(-kt)$$

Where Ct signifies the concentration of residual substrate at a specific time, core presents the initial substrate concentration, k is the degradation rate constant (day^{-1}), and $t_{1/2}$ represents the half-life

$$((t_{1/2} = \ln 2)/k)$$

BaP degradation experiments were conducted in duplicate, and average measurements were used for analysis. Statistical significance of observed variations was evaluated through one-way ANOVA using SPSS version 22. This analysis aimed to assess the meaningfulness of the observed differences in the experimental data.

RESULTS AND DISCUSSION

PAHs and the presence of PAH-degrading bacteria in mangrove sediments

The concentration of total PAHs in sediment samples varied from 230.8 ngg^{-1} to 754.7 ngg^{-1} when measured on a dry weight basis. The pattern of PAH composition within the mangrove sediments was primarily characterized by the prevalence of PAH components with Higher molecular weights (containing 4-6 rings). Notably, benzo[ghi]-perylene and indeno [1,2,3-cd] pyrene emerged as the predominant compounds across different sampling stations with in the Karankadu mangrove swamp (as outlined in **Table 1**). These recorded concentrations are unlikely to induce any detrimental impacts on the biological ecosystem, as they fall below the lower range of values associated with effects [27]. Furthermore, these concentrations remain lower than those observed in comparable mangrove sediment studies conducted worldwide, encompassing locations such as Puerto Rico [28], the Caribbean Islands [29]. The Jiu long River estuary [30], and Hong Kong [22]. However, an array of high molecular weight PAH compounds was uncovered, encompassing benzo(b)fluoranthene, diben(a,h)anthracene, indeno[1,2,3-cd] pyrene, and

benzo(ghi)perylene. Regrettably, the associated toxicity criteria for these compounds were not attainable, under scoring the need for further investigation. Additionally, **Table 1** illustrates the variation in Phe/Ant ratios within Karankadu sediments, ranging from 0.8 to 2.4. This range falls below the ratio of 10 specified by [31], indicative of contamination from a paralytic source. Conversely, the Flu/Pyr ratios (ranging from 0.19 to 0.89) remained below unity, implying a petrogenic origin of contamination. These observations collectively suggest that the PAHs present in Karankadu mangrove sediments emanated from a combination of both pyrolytic and petrogenic sources. Mangroves, the predominant wet lands found in subtropical and tropical coastal regions, possess the remarkable capacity of plants and microorganisms to modify and disintegrate hazardous so organic compounds, such as oil constituents [11]. This study focuses on bacterial consortia cultivated from diverse mangrove sediment samples, show casing abroad spectrum of potential for degrading complex. The capability of a microbial community to metabolize PAHs is contingent upon the composition and abundance of hydrocarbon-degrading microorganisms within the community, a composition influenced by prior exposure to aromatic hydro carbons through sustained or singular events [32,33]. Within contaminated sediments, it is hypothesized that bacterial strains possessing resistance to xenobiotic pollutants and displaying efficient decomposition capabilities are present [34]. Research shows that identified numerous bacterial strains with the capacity to mineralize diverse array of PAHs extracted from polluted sites [3]. Previous exposure and acclimatization of microorganisms to PAHs within sediments were also demonstrated to enhance the rates of breakdown [35]. The abundance of PAH-degrading bacteria within the Karankadu mangrove sediments varied significantly across different sampling stations. The quantities of phenanthrene-degrading bacteria spanned from 1.12×10^5 to 6.28×10^5 CFU g^{-1} dry weights (as detailed in **Table 2**). Pyrene-degrading bacteria ranged from 2.20×10^5 to 6.0×10^5 CFU g^{-1} dry weight. Similarly, fluoranthene-degrading bacteria exhibited arrange of 1.71×10^5 to 6.37×10^5vCFU g^{-1} dry weight, while benzo(a)pyrene-degrading bacteria ranged from 1.83×10^5 to 4.81×10^5 CFU g^{-1} dry weight. Notably, station 1 presented the lowest value among there corded data. In a comprehensive examination of bacterial diversity within PAH-contaminated regions, research [36] demonstrated that the ability of bacteria to degrade PAHs is linked to specific phylogenetically distinct genera. They also discussed the presence of bacteria capable of PAH degradation in uncontaminated water regions, although it remains uncertain whether PAH-degrading organisms can be isolated from uncontaminated soil sites. While our study observed lower individual and over all PAH concentrations compared too there developed areas, it was note worthy that mangrove sediments still hosted a substantial population of PAH-degrading bacteria. This observation suggests that various strains of PAH-degrading bacteria with significant potential for PAH degradation may be present within mangrove sediments. The bacterial community was extracted from mangrove sediments and effectively utilizes PAHs (phenanthrene, fluoranthene, pyrene, and benzo(a)pyrene) as exclusive carbon sources. Following an incubation period of 15–20 days, three promising isolates were distinguished based on their zone of inhibition and Optical density (OD) values. The resulting DNA sequences (PVS01:1478bp; PVS02:1442bp; PVS03:1502bp) were submitted for comparison with the16S rRNA gene database housed in Gene Bank-NCBI, assigned with accession numbers OQ954107-OQ954109 (as illustrated in **Figure 2**). The present stud further corroborates the efficacy of *B. cereus* in effectively breaking down benzo(a)pyrene (BaP) when utilized as the exclusive carbon source. **Figure 3** illustrates the biodegradation process of benzo(a)pyrene (BaP) carried out by three identified bacterial species over a period of 10 day under incubation conditions. Operating at approximately 30°C, *Bacillus cereus, Achromobacter insolitus*, and *Pseudomonas aeruginosa* effectively degraded 20 mg/L of BaP within a saline environment (28 ppm NaCl). Despite the inherent recalcitrance of BaP, the respective breakdown percentages achieved by *B. cereus*, *A. insolitus*, and *P. aeruginosa* were 37%, 34%, and 26%. Notably, a minor portion of BaP loss (7%) in the control group could be attributed to non-biological processes. This significant finding underscores the distinctive capability of each organism in terms of degrading BaP. Consequently, *B. cereus* exhibited a comparatively faster degradation rate, while *P. aeruginosa* displayed as lower rate of degradation. The outcomes of the statistical analysis (as presented in **Table 3**) indicated significant deviations of individual isolates from the initial concentration at a significance level of $p<0.05$. To assess the biodegradation rate of BaP by each individual isolate over a span of 10 days, a first-order kinetic model was employed. The outcomes revealed that *B. cereus, A. insolitus*, and *P. aeruginosa* displaye degradation rate constants of 0.046, 0.041, and 0.03 day^{-1}, respectively, resulting in half-lives of 15.1, 16.7, and 23.3 days. In contrast, the control group exhibited a rate constant of 0.007 and a half-life of 94.7 days. These findings unequivocally under scored the capacity of all three isolates to diminish BaP, with *B. cereus* demonstrating a more efficient reduction compared to *A. insolitus* and *P. aeruginosa*. strains proficient in the degradation of benzo(a)pyrene (BaP) encompass

Sphingomonas paucimobilis strain EPA 505 [37], *Mycobacterium vanbaalenii* PYR-1 [38], *Beijernickia* strain B836 [39], *Bacillus subtilis* [40]. *Sphingomonas yanoikuyae* JAR02 [41], *Sphingomonas* sp.VKMB-2434 [42], *Pseudomonas aeruginosa* PSA5 and *Rhodococcus* sp. NJ2 [43], as well as *Achromobacter* sp., *Marinobacter* sp., and *Rhodanobacter* sp.[44], *Pseudomonas aeruginosa* [45], and *Olleya* sp. ITB9 [46]. In a study [47], they were observed that after a 14-day incubation period, the *Ochrobactrum* sp. BL01 strain managed to degrade 21% of BaP, while the enhanced consortium achieved a significantly higher degradation rate of 44% for BaP. In contrast [48] reported that strain P14 achieved a degradation of 30% of BaP at a concentration of 50 mg/L over a 30-day incubation period. Machin-Ramirez et al. [49] uncovered that *B. mycoides* and *Pseudomonas* sp. achieved degradation rates ranging from 1% to 35% for BaP concentrations ranging from 25 mg/L to 75 mg/L, with a cultivation period of 5 days. Additionally, Mishra and Singh [43] demonstrated that *Pseudomonas aeruginosa* PSA5 and *Rhodococcus* sp. NJ2 achieved impressive degradation rates of 88% and 47%, respectively, for BaP within a 25-day time frame. In a comparative analysis between the bacteria isolated in our study and previously identified strains, it was revealed that *Bacillus cereus* achieved degradation of 37% BaP (20 mg/L) over the incubation period of 10 days. Similar results has been observed in thermophilic *Bacillus* strains, with a particular emphasis on *B. licheniformis* and *B. subtilis* [50,51,52]. Furthermore, *Bacillus* strains, including *B. cereus* have been noted to exhibit degradation capabilities for various other aromatic compounds as well [53-57]. Notably, among others *B. cereus* has also been documented as having the more capability to degrade BaP in saline environments. Among the myriad of aromatic hydro carbons prevalent in petrochemical waste, benzo[a]pyrene stands out as one of the most carcinogenic and perilous polycyclic aromatic hydrocarbons (PAHs). Subsequent investigations have revealed that bacteria have the capacity to degrade benzopyrene while thriving on a carbon source within a liquid culture medium experiment. In an experiment conducted by Ye et al. [58] a reduction of 5% in BaP concentration was observed after 168 hours of incubation with *Sphingomonas paucimobilis* strain EPA 505. Moreover, a variety of bacteria have been identified with the ability to metabolize benzopyrene. Notable examples include at least three *Pseudomonas* species, *Agrobacterium*, *Bacillus*, and *Sphingomonas* species. Additionally, benzopyrene metabolism Has been observed in other bacteria such as *Rhodococcus* sp. and *Mycobacterium*, along with certain mixed cultures involving *Pseudomonas* and *Flavobacterium* spp. BaP biodegradation was accomplished through an enhanced bacterial consortium within a saline environment (28 ppm NaCl), supplemented with 50 mg/L of BaP as the sole carbon and energy source. Under conditions of 30°C and 150 rpm agitation, the consortium demonstrated efficient BaP degradation within the medium. Over the incubation period, the consortium exhibited an initial rapid BaP degradation within the first four days, followed by a subsequent lower degradation rate. A minor portion of BaP (10%) was attributed to abiotic loss. The enhanced consortium successfully achieved a remarkable 44% degradation of BaP after 10-day duration. Substantiating this, statistical analysis (as presented in **Table 4**) indicated that the consortium's BaP degradation surpassed the base line BaP concentration at a significance level of $p<0.05$. This study effectively show cased that the utilization of a bacterial consortium led to a more enhanced breakdown of BaP compared to individual bacterial cultures. The kinetic degradation analysis of BaP by the bacterial consortium (B+A+P) (as outlined in **Table 4**) yielded a degradation rate constant (k) of $0.05 day^{-1}$, resulting in a half-life of 11.9 days. In contrast, the control group exhibited a degradation reaction rate of $0.011\ day^{-1}$ and a corresponding half-life of 81.2 days. The capability of the consortium to breakdown BaP was assessed, and it show cased its proficiency under optimal conditions of 35°C, with a saline solution containing 1.5% NaCl, and at a neutral pH level. The meticulously defined bacterial consortium accomplished the degradation of BaP at a rate of 2.57 mg/L. day, which significantly surpassed the rates reported in previous studies 0.55 mg/L. day [59], 0.31 mg/L. day [47] and 2.3 mg/L. day [60]. The observed disparities in pollutant reduction across the microbial consortia investigated in the studies mentioned earlier and the current research might be attributed to variations in initial concentrations and the specific isolates utilized. Furthermore, the outcomes presented in this study exceeded those achieved by pure cultures, such as *Klebsiella pneumonia* PL1 at a rate of 0.57 mg/L. day [61], *Rhodococcus* sp. P14 at 0.5 mg/L. day [48], *Porphyrobacter* sp at 1.2 mg/L.day [62], *Burkholderia cepacia* at 0.40 mg/L. day [63] and *Ochrobactrum* sp. BL01. Indeed Mohandass [64] discovered that a consortium composed of *B. cereus* and *B. vireti* managed to degrade 500 mg/L of BaP by 59% within a span of 35 days. Similarly, Pugazhendi [65] reported successful biodegradation of high molecular weight (HMW) PAHs through enriched bacterial consortia. Isaac et al. [66] also highlighted the potential of mixed cultures in eliminating HMW PAHs, demonstrating their efficacy in comparison to pure strains. In various studies, *Bacillus* spp., notably *B. cereus*, have demonstrated the ability to degrade benzo(a)pyrene (BaP). For instance, investigations have highlighted the BaP degradation potential of a consortium comprising *B. cereus* and *B. vireti* [64]. This suggests that the collective metabolism facilitated

by bacterial consortia may indeed accelerate the degradation of BaP. Metabolites generated by individual isolates have the potential to serve as significant substrates for the catabolism and growth of other species through intermediary biotransformation processes [67]. As per numerous microbial degradation research studies, the optimal pH range for PAH breakdown falls between 6 and 8, with neutrality being the most common value [68, 69]. However, it's worth noting that PAHs can also effectively degrade in both acidic and alkaline environments. Upto now, investigations on active bacteria degrading BaP have predominantly focused on the pH 7(**Figure 4**) range. In terms of temperature, it has been observed that several thermophilic bacterial species are capable of PAH breakdown within the 50 to 70°C [70]. Guevara-Luna et al. [52] reported that to date, only one bacterial strain, the thermophilic *Bacillus licheniformis* M2-7, has been identified as digesting BaP as its sole substrate at 50°C. In connection with salinity, within the realm of halotolerance, it's established that *Staphyloccocus* genus members can thrive in environments with 7.5–10% NaCl) [71, 72]. Arulazhagan et al. [44] reported, a cluster of bacteria, including *Achromobacter* sp. AYS3, *Marinobacter* sp. AYS4, and *Rhodanobacter* sp. AYS5, has been utilized to degrade BaP under saline conditions via co-metabolism (in the presence of phenanthrene) within a medium containing 3-9% NaCl. Recent findings highlight the capability of *Ochrobactrum anthropi*, *Stenotrophomonas acidaminiphila*, and *Aeromonas salmonicida* to degrade BaP in seawater, which typically holds a salt level of around 3-5% NaCl [25]. However, the current investigation did not establish a significant correlation between the total concentrations of (PAHs) within mangrove sediments and the capability of the enhanced microbial consortia to degrade these PAHs. In a prior study [73] also noted that the biodegradation percentages of phenanthrene-degrading bacterial consortia did not exhibit an apparent linkage to the degree of PAH contamination. Similarly, Grosser et al. [1] identified that elevated levels of microbial activity in soil did not exert a notable impact on PAH mineralization. Notably, the extent of PAH metabolism within soil appeared to be independent of microbial community activity, with its modulation being significantly influenced by various other soil properties. Discrepancies in the capacity for PAH degradation observed among consortia enriched from distinct sediments can be ascribed to the unique physical and chemical attributes of sediments within each mangrove swamp. Additionally, the configuration of the native microbial community, particularly the composition and abundance of microorganism's proficient in the degradation of hazardous pollutants, is a crucial determinant.

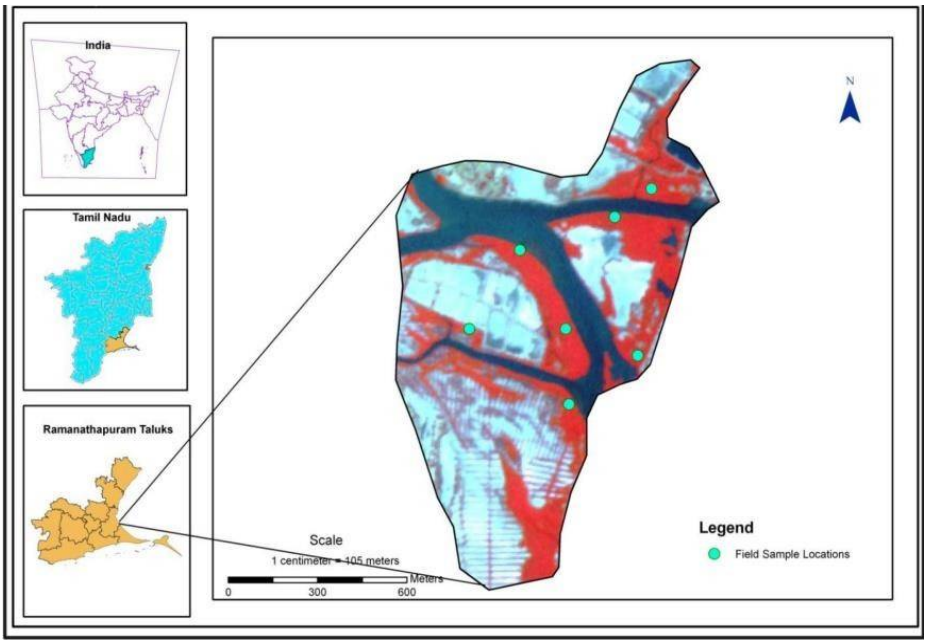

Figure 1. Shows the geographical location of Karankadu mangrove and the different types of sampling collection areas.

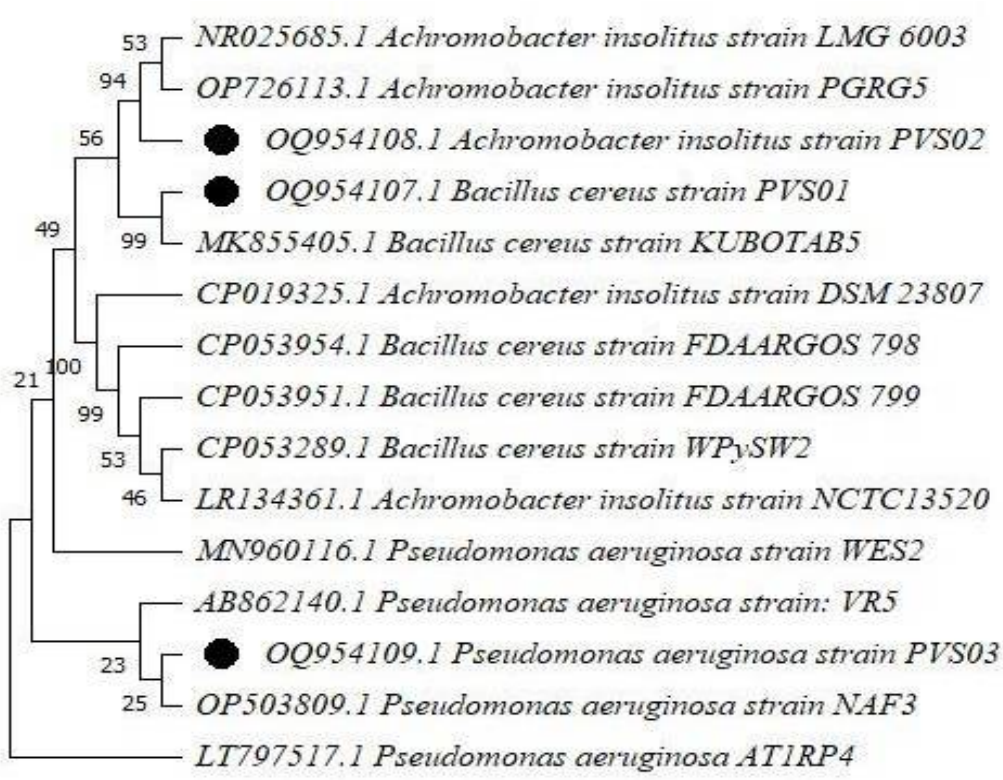

Figure 2. Shows phylogenetic relationships among three prospective PAHs degrading bacteria using a 16S rDNA Gene Sequence

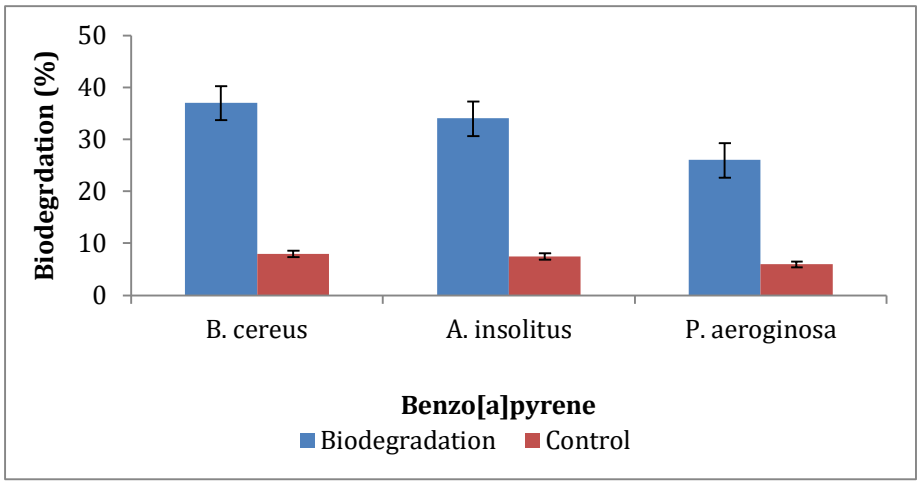

Figure 3. Degradation of 20 mg/L BaP by *B. cereus, A. insolitus* and *P. aeroginosa*

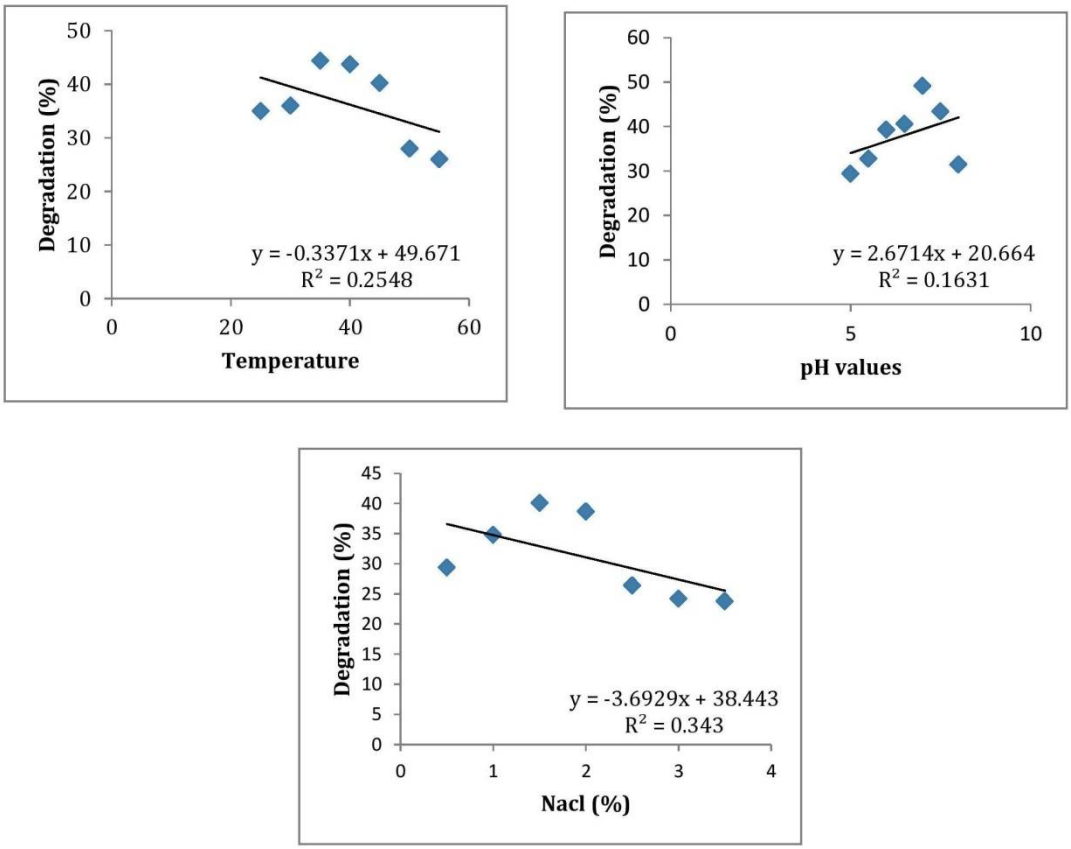

Figure 4. Optimization of BaP degradation by Consortium inoculum with various, temperature, pH and NaCl conditions.

Table 1. Concentrations of total PAHs and model PAHs, phenanthrene (Phe), fluoranthene (Flu), pyrene (Pyr) and benzo(a)pyrene (BaP), percentages of HMW-PAH to total and ratios of phenanthrene/anthracene (Phe/Ant) and fluoranthene/pyrene (Flu/Pyr) in sediment samples collected from the Karankadu mangrove

	PAH Concentration ng g^{-1} dw					Specific PAH ratio	
Sediments	**Total**	**Phe**	**Flu**	**Pyr**	**Ba Py**	**Phe/Ant**	**Flu/Pyr**
Station 1	754.7	6.3	23.2	51.9	25.3	2.2	0.45
Station 2	616.4	7.1	27.2	30.4	23.2	2.4	0.89
Station 3	214.7	5.4	21.2	25.3	10.7	2.2	0.84
Station 4	386.4	1.8	19.3	104.1	9.2	0.8	0.19
Station 5	324.9	4.7	22.3	59.7	30.3	1.01	0.37
Station 6	740.3	6.1	31.4	64.3	45.4	2.1	0.49
Station 7	230.8	2.2	18.4	81.4	22.7	0.9	0.23
ER-L[a]	4022	2404	600	665	430	NA	NA[c]
ER-M[a]	44792	1500	5100	2600	1600	NA	NA[c]

a. ER-L, effects range-low; ER-M, effects range-median (Long et al. 1995).
b. HMW-PAHs(high molecular weight-PAHs) including fluoranthene, pyrene, benzo(a)anthracene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-c,d)pyrene and benzo(g,h,i)perylene.
c. NA, not available.
d. NAc, not calculated due to the concentration of individual PAH compound being below the detection limits.

Table 2. PAH-degrading bacteria (CFU g $^{-1}$ dry weight) in mangrove sediments

Sediments	Phe-degrading bacteria	Pyr-degrading bacteria	Flu-degrading bacteria	BaP-degrading bacteria
Station 1	1.12 ×10^5	2.20 ×10^5	1.71 ×10^5	2.38 ×10^5
Station 2	3.58×10^5	5.25 ×10^5	1.83 ×10^5	4.37 ×10^5
Station 3	5.62 ×10^5	5.1 ×10^5	4.72 ×10^5	3.68 ×10^5
Station 4	6.28 ×10^5	5.9 ×10^5	6.37 ×10^5	4.81 ×10^5
Station 5	5.1 ×10^5	4.7 ×10^5	4.1 ×10^5	3.25 ×10^5
Station 6	4.74 ×10^5	3.1 ×10^5	5.3 ×10^5	1.83 ×10^5
Station 7	3.89 ×10^5	6.0 ×10^5	4.3 ×10^5	4.32 ×10^5

Table 3. Degradation kinetics of BaP under single isolates after 10 days

PAH	Single Isolates	Degradation %	Rate of Bap degradation k (day^{-1})	Half-life (ln2/k) (days)
BaP (n=3)	*B. cereus*	37	0.046	15.1
BaP (n=3)	*A. insolitus*	34	0.041	16.7
BaP (n=3)	*P. aeroginosa*	26	0.03	23.3
BaP (n=3)	Control	7	0.007	94.7

*n= no of replicate

Table 4. Degradation of BaP under single isolates after 10 days

PAH	Consortium	Degradation %	Rate of Bap degradation k (day^{-1})	Half-life (ln2/k) (days)
BaP (n=3)	B+A+P	44	0.05	11.9
BaP (n=3)	Control	10	0.011	81.2

*n= no of replicate

*B = *Bacillus cereus*: A = *A. insolitus*: P = *P. aeroginosa*

CONCLUSION

Mangrove sediments play host to a diverse array of microorganisms, each possessing distinct potential for the degradation of mixed PAHs (including phenanthrene, fluoranthene, pyrene, and benzo(a)pyrene). Intriguingly, the enriched microbial consortia's capacity to degrade PAHs did not exhibit a direct correlation with the extent of PAH contamination within the sediments. This study sheds light on the remarkable proficiency of the examined bacterial consortia in efficiently breaking down benzo[a]pyrene (BaP), in saline conditions. Notably, the bacterial consortium demonstrated a BaP degradation capability on par with that of pure cultures. Among the identified bacterial strains, namely *Bacillus cereus*, *Achromobacter insolitus*, and *Pseudomona aeruginosa*, all known for their PAH-degrading abilities, variations in degradation percentages were observed. Furthermore, the study unveiled that the bacterial consortium's optimal conditions for BaP breakdown were at a temperature of 35°C, a pH of 7.0, and a saline concentration of 1.5% NaCl. Significantly, this research uncovered the previously unrecognized microbiological prowess of *Bacillus cereus*, *Achromobacter insolitus*, and *Pseudomonas aeruginosa* in BaP degradation. These findings highlight that mangrove surface sediment serve as promising reservoirs of PAH-degrading microorganisms, which can be harnessed for PAH bioremediation purposes, both as part of a consortium and as individual isolates.

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CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

AUTHOR'S CONTRIBUTION

The authors confirmed their contributions to the paper as follows: Study conception, design & sample collection by author PJ. Data collections: author JR. Analysis and interpretation of results: authors GK &KJ. Draft manuscript preparation: authors PJ & JR. All authors reviewed the results and approved the final

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REFERENCES

1. Grosser, R.J., Warshawsky, D. & Vestal, J.R. (1995). Indigenous and enhanced mineralization Pyrene, Benzo[a]pyrene, and carbazolde in soils. Appl. Environ. Microbiol., 57:3462-3469.
2. Chaineau, C.H., Morel, J., Dupont, J., Bury, E. & Oudot, J. (1999). Comparison of the fuel oil Biodegradation potential of hydrocarbon-assimilating microorganisms isolated from a temperate agriculture soil. Sci. Total. Environ., 227:237-247.
3. Aitken, M.D., Stringfellow, W.T., Nagel, R.D, Kazunga, C.U. & Chen, S.H. (1998). Characteristics of phenanthrene-degrading bacteria isolated from soils contaminated with polycyclic aromatic hydrocarbons. Can. J. Microbiol., 44:743–752.
4. Jesus, F., Pereira, J.L., Campos, I., Santos, M., Re, A., Keizer, J., Nogueira, A., Gonçalves, F.J., Abrantes, N. & Serpa, D. (2022). A review on polycyclic aromatic hydrocarbons distribution in freshwater ecosystems and their toxicity to benthic fauna. Sci. Total. Environ., 820:153282.
5. Ostrem Loss, E.M. & Yu, J.H. (2018). Bioremediation and Microbial Metabolism of Benzo(a)Pyrene. Mol. Microbiol., 109:433-444.
6. Prasanna, J., Seshapriya, Ve., Anand, M., Kumaraguru A.K. & Rangesh, K. (2017). Biodiversity assessment of crabs, gastropods and bivalves in Chinnapalam creek of the southeast coast of India. Indian J. Mar. Sci., 46(9):1751-1757.
7. Alongi, D.M. (2002). Present state and future of the world's mangrove forests. Environ. Conserv., 29:331–349.
8. Santos, H.F., Carmo, F.L., Paes J.E.S., Rosado, A.S. & Peixoto, R.S. (2011). Bioremediation of mangroves impacted by petroleum. Water Air Soil Pollut., 216:329-350.
9. Ke, L., Wong, T.W.Y., Wong, Y.S. & Tam, N.F.Y. (2002). Fate of polycyclic aromatic hydrocarbon (PAH) contamination in a mangrove swamp in Hong Kong following an oil spill. Mar. Pollut. Bull., 45:339-347.
10. Alongi, D.M. (1988). Bacterial productivity and microbial biomass in tropical mangrove sediments. Microbiol. Ecol., 15:59-79.
11. Ramsay, M.A., Swannells, R.P.J., Shiton, W.A., Duke, N.C. & Hill, R.T. (2000). Effect of bioremediation on the microbial community in oiled mangrove sediments. Mar. Pollut. Bull., 41:413-419.
12. Yu, S.H., Ke, L., Wong, Y.S. & Tam, N.F.Y. (2005). Degradation of polycyclic aromatic hydrocarbons (PAHs) by a bacterial consortium enriched from mangrove sediments. Environ. Int., 31:149-676.
13. Nzila, A. (2018). Current status of the degradation of aliphatic and aromatic petroleum hydrocarbons by thermophilic microbes and future perspectives. Int. J. Environ. Res. Publ. Health., 15:2782.
14. Ghosal, D., Ghosh, S., Dutta, T.K. & Ahn, Y. (2016). Current state of knowledge in microbial degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. Front. Microbiol., 7:1369.
15. Lawal, A.T. (2017). Polycyclic Aromatic Hydrocarbons: A Review. Cogent Environ. Sci., 3:1339841.
16. de Lima, R.F., Dionello, R.G., Peralba, M.D.C.R., Barrionuevo, S., Radunz, L.L. & Junior, F.W.R. (2017). PAHs in corn grains submitted to drying with firewood. Food Chem., 215:165-170.
17. Fatma Aygun, S. & Kabadayi, F. (2005). Determination of Benzo[a]pyrene in charcoal grilled meat samples by HPLC with fluorescence detection. Int. J. Food. Sci. Nutr., 56:581-585.
18. Ramesh, A., Harris, K.J. & Archibong, A.E. (2017). Reproductive Toxicity of polycyclic aromatic hydrocarbon (Eds. Gupta, R.C.) Reproductive and developmental toxicology. Academic Press, USA, p.745-763.
19. Gray, J.P. (2014). Benzo(a)pyrene (Eds. Wexler P) Encyclopedia of toxicology. Academic Press, Cambridge, MA, USA, p.423-428.
20. Prasanna, J., Anand, M., Vijayasekaran, D. & Kumaraguru A.K. (2017). Allometric model for estimating above ground biomass and carbon storage in Karankadu mangrove swamp, Palkbay, Southeast coast of India. Indian J. Mar. Sci., 46(8):1682-1692.
21. Sankarappan, R., Gopalakrishnan, G., Shanmugam, R., Magalingam, R., Chockalingam, K. & Ayyamperumal, R. (2021). Diffusion, textural characteristics, and source identification of the heavy metals in the Karankadu mangrove sediments, South India. Arab. J. Geosci., 14(4):329.
22. Tam, N.F.Y., Ke, L., Wang, X.H. & Wong, Y.S. (2001). Concentration of polycyclic aromatic Hydrocarbons in surface sediments of mangrove swamps. Environ. Pollut., 114:255-263.
23. Kastner, M. & Mahro, B. (1994). Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons. Appl. Microbiol. Biotechnol., 41:267-273.
24. Ikenaga, M., Muraoka, Y., Toyota, K. & Kimura, M. (2002). Community structure of the Microbiota associated with nodal roots of rice plants along with the growth: Estimation by PCR-RFLP analysis. Biol. Fertil., 36:405-410.
25. Aziz, A., Agamuthu, P., Alaribe, F.O. & Fauziah, S.H. (2018). Biodegradation of benzo[a]pyrene by Bacterial consortium isolated from mangrove sediment. Environ. Technol., 39(4):527-535.
26. Alaribe, F. & Agamuthu, P., (2015). Assessment of phytoremediation potentials of Lantana Camara in Pb impacted soil with organic waste additive. Ecol. Eng., 83:513-520.
27. Long, E.R., MacDonald, D.D., Smith, S.L. & Calder, F.D. (1995). Incidence of adverse biological effects with in ranges

of chemical concentrations in marine and estuarine sediments. Environ. Manage., 19:81-97.
28. Klekowski, J., Corredor, J.E., Morell, J.M. & Dolcastello, C.A. (1994). Petroleum pollution and Mutation in mangroves. Mar. Pollut. Bull., 28:166-169.
29. Bernard, D., Pascaline, H. & Jeremie, J.J. (1996). Distribution and origin of hydrocarbons in sediments from lagoons with fringing mangrove communities. Mar. Pollut. Bull., 32:734-739.
30. Tian, Y., Liu, H.J., Zheng, T.L., Kwon, K.K., Kim, S.J. & Yan, C.L. (2008). PAHs contamination and bacterial communities in mangrove surface sediments of the Jiulong River Estuary, China. Mar. Pollut. Bull., 57:707-715.
31. Soclo, H.H., Garrigues, P.H. & Ewald, M. (2000). Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas. Mar. Pollut. Bull., 40:387-396.
32. Heitkamp, M.A. & Cerniglia, C.E. (1987). Effects of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems. Environ. Toxicol. Chem., 6:535-546.
33. Garcia-Valdes ,E., Cozar, E., Rotger, R., Lulucat, J. & Ursing, J. (1988). New naphthalene degrading marine *pseudomonas* strains. Appl. Environ. Microbiol., 54:2478-248
34. Koutny, M., Ruzicka, J. & Chla chula, J. (2003). Screening for phenol degrading bacteria in the pristine soils of south siberia. Appl. Soil. Ecol., 23:79-83.
35. Wilson, S.C. & Jones, K.C. (1993). Bioremediation of soil contaminated with polynuclear aromatic Hydrocarbons (PAHs): A review. Environ. Pollut., 81:229-249.
36. Mueller, J.G.R., Devereux, D.L, Santavy, S.E., Lantz, S.G. & Willis, P.H. (1997). Phylogenetic and physiological comparisons of PAH-degrading bacteria from geographically diverse soils. Antoine Van Leeuwen hoek Int. J. Gen. Mol. Microbiol., 71:329-343.
37. Ye,D., Siddiqi, M.A., Maccubbin, A.E., Kumar, S. & Sikka, H.C. (1995). Degradation of polynuclear aromatic hydrocarbons by *Sphingomonas paucimobilis*. Environ. Sci. Technol., 30:136-142.
38. Moody, J.D., Freeman, J.P., Fu, P.P. & Cerniglia, C.E. (2004). Degradation of benzo[a]pyrene by *Mycobacterium vanbaalenii* PYR-1. Appl. Environ. Microbiol., 70:340-345.
39. Gibson, D.T, Mahadevan, V., Jerina, D.M., Yogi, H. & Yeh, H. (1975). Oxidation of the carcinogens benzo[a]pyrene and benzo[a]anthracene to dihydrodiols by a bacterium. Science., 189:295-297.
40. Hunter, R.D., Ekunwe, S.I., Dodor, D.E., Hwang, H.M. & Ekunwe, L. (2005). *Bacillus subtilis* is a potential degrader of pyrene and benzo[a]pyrene. Int. J. Environ. Res. Publ. Health., 2,267-271.
41. Rentz, J.A., Alvarez, P.J. & Schnoor, J.L. (2008). Benzo[a]pyrene degradation by *Sphingomonas yanoikuyae* J AR02. Environ. Pollut ., 151:669-677.
42. Baboshin, M., Akimov, V., Baskunov, B., Born, T.L., Khan, S.U. & Golovleva, L. (2008).Conversion of Polycyclic aromatic hydrocarbons by *Sphingomonas* sp. VKM 2434. Biodegradation., 19:567–576.
43. Mishra, S, & Singh, S. (2014). Biodegradation of benzo(a)pyrene mediated by catabolic enzymes of bacteria. Int. J. Environ. Sci. Technol., 11:1571-1580.
44. Arulazhagan, P., Sivan, C., Kumar, S.A., Aslam, M. & Banu, J.R. (2014). Co-metabolic degradation of benzo(e)pyrene by halophilic bacterial consortium at different saline conditions. J. Environ. Biol., 35:445-452.
45. Waszak, D.Q., da Cunha, A.C.B., Agarrallua, M.R., Goebel, C.S. & Sampaio, C.H. (2015). Bioremediation of a Benzo [a] Pyrene-contaminated soil using a microbial consortium with *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus flavus*, and *Fusarium* sp. Water Air Soil Pollut., 226:1-11.
46. Okai, M., Watanabe, A., Ishida, M. & Urano, N. (2015). Draft genome sequence of a Benzo[a] pyrene-degrading dacterium, *Olleya* sp. strain I TB9. Genome Announ., 3(6):10-1128.
47. Luo, Y., Tian, Y., Huang, X., Yan, C., Hong, H., Lin, G. & Zheng, T. (2009). Analysis of community Structure of a microbial consortium capable of degrading benzo(a)pyrene by DGGE. Mar. Pollut. Bull., 58:115-1163.
48. Song, X., Xu, Y., LiG Zhang, Y., Huang, T. & Hu, Z. (2011). Isolation, characterization of *Rhodococcus* sp. P14 capable of degrading high-molecular-weight polycyclic aromatic hydrocarbons and aliphatic hydrocarbons. Mar. Pollut. Bull., 62:2122-2128.
49. Machin-Ramirez, C., Morales, D., Martinez-Morales, F., Okoh, A. & Trejo-Hernandez, M. (2010). Benzo[a]pyrene removal by axenic and co-cultures of some bacterial and fungal strains. Int. Biodeterior. Biodegrad., 64:538-544.
50. Zhao, Z. & Wong, J.W.C. (2010). Rapid biodegradation of Benzo[a]pyrene by *Bacillus subtilis* BUM under thermophilic condition. Environ. Eng. Sci., 27:939-945.
51. Feitkenhauer, H., Muller, R. & Markl, H. (2003). Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 60–70 degrees by *Thermus* and *Bacillus* spp. Biodegradation., 14:367-372.
52. Guevara-Luna, J., Alvarez-Fitz, P., Ríos-Leal, E., Acevedo-Quiroz, M., Encarnacion-Guevara, S., Moreno-Godinez, M.E., Castellanos-Escamilla, M., Toribio-Jiménez, J. & Romero-Ramirez, Y. (2018). Biotransformation of benzo[a]pyrene by the thermophilic bacterium *Bacillus licheniformis* M2-7. World J. Microbiol. Biotechnol., 34:1-9.
53. Seo, J., Keum, Y & LiQ, X. (2009). Bacterial degradation of Aromatic compounds. Int. J. Environ. Res. Publ. Health., 6:278-309.
54. Haritash, A.K. & Kaushik, C.P. (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A Review. J. Hazard Mater., 169:1-15.
55. Tarafdar, A., Sinha, A. & Masto, R.E. (2017). Biodegradation of anthracene by a newly isolated bacterial strain, *Bacillus thuringiensis* AT. ISM.1, Isolated from a fly ash deposition Site. Lett. Appl. Microbiol., 65:327-334.
56. Doddamani, H.P. & Ninnekar, H.Z. (2000). Biodegradation of Phenanthrene by a *Bacillus* species. Curr. Microbiol., 41:11-14.

57. Bibi, N., Hamayun, M., Khan, S.A., Iqbal, A., Islam, B., Shah, F., Khan, M.A. & Lee, I.J. (2018). Anthracene biodegradation capacity of newly isolated rhizospheric bacteria *Bacillus cereus* S13. PLoS One, 13(8):e0201620.
58. Ye,D., Siddiqi, M.A., Maccubbin, A.E., Kumar ,S. & Sikka, H.C. (1995). Degradation of polynuclear aromatic hydrocarbons by *Sphingomonas paucimobilis*. Environ. Sci. Technol., 30:136-142.
59. Thavamani, P., Megharaj, M. & Naidu, R. (2012). Bioremediation of high molecular weight polyaromatic hydrocarbons co-contaminated with metals in liquid and soil slurries by metal tolerant PAHs degrading bacterial consortium. Biodegradation., 23:823-835.
60. Sun, R., Jin, J., Sun, G., Liu, Y. & Liu, Z. (2010). Screening and degrading characteristics and community structure of a high molecular weight polycyclic aromatic hydrocarbon degrading bacterial consortium from contaminated soil. J. Environ. Sci. 22:1576-1585.
61. Ping, L., Zhang, C., Zhang, C., Zhu, Y., He, H., Wu, M., Tang, T., Li, Z. & Zhao, H. (2014). Isolation and characterization of pyren and benzo[a]pyrene degrading *Klebsiella pneumonia* PL1 and its potential use in bioremediation. Appl. Microbiol. Biotechnol., 98:3819-3828.
62. Gauthier, E., Deziel, E., Villemur, R., Juteau, P., Lepine, F. & Beaudet, R. (2003). Initial characterization of new bacteria degrading high molecular weight polycyclic aromatic hydrocarbons isolated from a 2-year enrichment in a two liquid phase culture system. J. Appl. Microbiol., 94:301-311.
63. Juhasz, A.L, Britz, M.L. & Stanley, G.A. (1997). Degradation of fluoranthene, pyrene, benz[a] anthracene and dibenz[a,h]anthracene by *Burkholderi cepacia*. J. Appl. Microbiol. 83:189-198.
64. Mohandass, R., Rout, P., Jiwal, S. & Sasikala, C. (2012). Biodegradation of Benzo[a]pyrene by the mixed culture of *Bacillus cereus* and *Bacillus vireti* isolated from the petrochemical industry. J. Environ. Bio., 33:985-989.
65. Pugazhendi, A., Abbad Wazin, H., Qari, H., Basahi, J.M.A.B, Godon, J. & Dhavamani, J. (2017). Biodegradation of low and high molecular weight hydrocarbons in petroleum refinery waste water by a thermophilic bacterial consortium. Environ. Technol., 38(19):2381-2391.
66. Isaac, P., Martinez, F.L., Bourguignon, N., Sanchez, L.A. & Ferrero, M.A. (2015). Improved PAHs removal performance by a defined bacterial consortium of indigenous *Pseudomonas* and a actinobacteria from Patagonia, Argentina. Int. Biodeterior. Biodegrad., 101:23-31.
67. Johnsen, A.R., WickL, Y. & Harms, H. (2005). Principles of microbial PAH-degradation in soil. Environ. Pollut., 133:71-84.
68. Margesin, R & Schinner, F. (2001). Biodegradation and bioremediation of hydrocarbons in Extreme environments. Appl. Microbiol. Biotechnol., 56:650-663.
69. Leahy, J.G. & Colwell, R.R. (1990). Microbial degradation of hydrocarbons in the environment. Microbiol. Rev., 54:305-315.
70. Nzila, A. (2018). Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons under anaerobic conditions: Overview of studies, proposed pathways and future perspectives. Environ. Pollut., 239:788-802.
71. Ventosa, A., Nieto, J.J. & Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev., 62:504-544.
72. Kim, H.J & Oh, S.W. (2010). Performance comparison of 5 selective media used to detect *Staphylococcus aureus* in foods. Food Sci. Biotechnol., 19:1097-1101.
73. Tam, N.F.Y., Guo, C.L., Yau, W.Y. & Wong, Y.S. (2002). Preliminary study on biodegradation of phenanthrene by isolated from mangrove sediments in Hong Kong. Mar. Pollut. Bull., 41(1-12):316-324.

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