IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ISOLATION AND CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBON DEGRADING ORGANISM FROM TIRUPUR CITY DUMP YARD SOIL

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ABSTRACT:

This study aims to isolate and characterize microorganisms that are capable of degrading polycyclic aromatic hydrocarbons (PAHs) from soil samples collected from the dump yard in Tirupur City, an industrial area known for its significant PAH contamination. Using enrichment culture techniques, bacterial strain was isolated. Minimal media with Naphthalene as a carbon source was used to isolate bacteria that exhibited the ability to utilize PAH as their sole carbon and energy source. Morphological, biochemical and molecular methods were performed to identify the isolated strains. Findings revealed that the isolated strains were *Sphingomonas paucimobilis* which was known for its hydrocarbon-degrading capabilities. This bacterial strain was then grown on different concentrations to evaluate the degradation ability. These strains demonstrated significant PAH degradation efficiency, highlighting their potential application in bioremediation strategies for PAH-contaminated sites. This study provides a valuable insight into the microbial diversity present in PAH-contaminated soils and underscores the importance of bioremediation as a sustainable approach for mitigating environmental pollution.

KEYWORDS: Polycyclic Aromatic Hydrocarbon (PAH), PAH contamination, hydrocarbon degradation, *Sphingomonas paucimobilis.*

INTRODUCTION:

Polycyclic Aromatic Hydrocarbons, or PAHs, are the largest group of cancer causing chemicals and are ninth in the list of chemical compounds that pose a risk to humans. While PAHs have been primarily studied for their carcinogenic properties, many PAHs are also genotoxic and mutagenic compounds, as well as teratogenic and carcinogenic compounds. PAHs are bio-accumulated in the tissues of living cells. Many PAHs are not carcinogenic per se, but act as synergists. Carcinogenicity of PAHs is related to the ability of PAHs to bind DNA, which causes a number of disruptive pathways that can lead to tumor initiation (Okechukwu Clinton Ifegwu *et al..*,2015). Any structural attribute or alteration of PAH molecules that increases DNA cross-linking can be carcinogenic. PAHs are composed of two or more fused aromatic rings composed of carbon and hydrogen atoms . They are typically created by incomplete combustion of organic materials, the release of petroleum products from fossil fuels, and a variety of industrial and domestic processes . PAHs can spread widely in the air, water, soil, and sediment after they are released. Soil is the primary sink for polycyclic aromatic hydrocarbons (PAHs) in the natural environment because of its hydrophobicity and lipophilicity (Nurela Ailijiang *et al.., 2022*). Roughly 90% of PAHs have been found to be able to be stored in soil. Through precipitation and surface runoff, PAHs in soils can enter surface and ground water, be released into the atmosphere by volatilization, and enter crops from contaminated soil and air through root and leaf adsorption.

Soil is an important environmental matrix to support the life of all organisms directly or indirectly. Despite being the ultimate sink for all pollutants, it has been neglected for long, which has negatively affected the quality of the soil. Disposal of pollutants has resulted in changes in properties of soils and introduction of toxicity into it (Abdel-Shafy *et al.., 2016*). The presence of heavy metals, pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons (PAHs) affects all forms of life since these chemicals have associated toxicity, mutagenicity, and carcinogenicity. PAHs are typical pollutants of soil which result in alteration in grain size, porosity and water-holding capacity of soil and affect diversity/population of microbes adversely. Significant changes in permeability, volume, plasticity, etc., are also brought about resulting in poor quality of contaminated soils. Considering the toxicity and global prevalence of PAHs, remediation of contaminated soils has become a challenge. Therefore, it is important to understand the detailed mechanism of physical, chemical or biological changes in soil (Ivana Jakovljevic *et al.., 2020*). Increased incidences of lung, skin, and bladder cancers are associated with occupational exposure to PAHs. Epidemiologic reports of PAH-exposed workers have noted increased incidences of skin, lung, bladder, and gastrointestinal cancers. These reports, however, provide only qualitative evidence of the carcinogenic potential of PAHs in humans because of the presence of multiple PAH compounds and other suspected carcinogens (Manthar Ali Mallah *et al.., 2022*). Airborne particles are able to transport the potentially mutagenic and carcinogenic compounds adsorbed to their surfaces, such as polycyclic aromatic hydrocarbons (PAHs). As a result, they can affect human health and, consequently, the quality of life. In the present paper, we analyzed household airborne particles and estimated the human health risk due to

PAH inhalation (M.T. Montano Soto *et al.., 2014*).

Human exposure to PAHs may occur from inhalation, dermal exposure or the ingestion of food contaminated with PAHs. PAHs in air pollution are primarily found bound to particulate matter; when PAHs are present in the gas phase, they have a duration of less than a day. Overall, the present scientific evidence suggests that the PAHs in ambient air are associated with increased cancer incidence in exposed populations (Narges Shamsetini *et al.., 2022*). Positive associations have been reported between ambient PAHs and breast cancer, childhood cancers and lung cancer. Epidemiological studies have shown that PAHs are associated with reduced lung function, exacerbation of asthma, and increased rates of obstructive lung diseases and cardiovascular diseases (Fernando Barbosa Jr. *et al.., 2023*).

Several pilot treatments have been implemented to prevent economic consequences and deterioration of soil and water quality. As a promising option, fungal enzymes are regarded as a powerful choice for degradation of PAHs. *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Bjerkandera adusta* are most commonly used for the degradation of such compounds due to their production of ligninolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase. The rate of biodegradation depends on many culture conditions, such as temperature, oxygen, accessibility of nutrients and agitated or shallow culture. Moreover, the addition of bio- surfactants can strongly modify the enzyme activity (Yanhua Qui *et al.., 2019*). Simultaneously, it becomes pertinent to identify the environmentally sustainable treatment options for remediation of contaminated sites. Whereas physical and chemical treatment methods are either cost, chemical, or energy prohibitive, the biological treatment is emerging as an efficient and effective option which employs microorganisms for mitigation (Hiaxuan Zhou *et al.., 2023*).

The removal of PAHs is dependent on the ionization potential. Degradation studies in soil are much more complicated than liquid cultures because of the heterogeneity of soil, thus, many factors should be considered when studying soil bioremediation, such as desorption and bioavailability of PAHs. Bacteria are the class of microorganisms actively involved in the degradation of organic pollutants from contaminated sites. A number of bacterial species are known to degrade PAHs. Most of them, representing biodegradation efficiency, are isolated from contaminated soil or sediments (Sandeep Bisht *et al.., 2015*). The organic pollutants which are in prolonged contact of the soil are bound to the soil particles and show reduced bioavailability towards biodegradation. The phenomenon is known as sequestration (Fazel Mohammadi- Moghadam *et al.., 2022*).

MATERIALS AND METHODS:

COLLECTION OF SAMPLES:

The collection of soil samples from the Tirupur city dump yard was conducted following a systematic and rigorous approach to ensure representative sampling and minimize contamination risks. Prior to sampling, all equipment, including stainless steel shovels, soil augers, and sampling containers, was thoroughly cleaned and sterilized to prevent cross-contamination between sampling points. Disposable gloves and masks were worn during the sampling process to minimize personal contamination. At designated sampling point, soil samples were collected from a depth of 15-30 cm, using stainless steel shovels. After collection, each soil sample was carefully placed into labeled, airtight sampling containers to prevent moisture loss and preserve the integrity of the samples (Besufekad *et al.., 2020)*.

Figure:1 Soil sample collected from city dump yard soil

SERIAL DILLUTION:

Serial dilution was performed to obtain bacterial cultures with manageable concentrations for subsequent analyses. 1gm of the soil sample obtained from the city dump yard and was suspended in 100ml of sterile distilled water, to create an initial 10^{-1} dilution. Subsequently, a series of sequential dilutions was carried out, including dilutions of 10^{-2}, 10^{-6}. For each dilution step, an aliquot of the previous dilution was transferred to fresh diluent and thoroughly mixed to ensure homogeneity. This process resulted in a gradual decrease in bacterial concentration across the dilution series (Ben David *et al..*, 2014).

GROWTH ON LURIA BERTANI AGAR:

The Luria Bertani agar was prepared and sterilized and was poured onto sterile petri plates. 100 µL of each dilution was spread evenly over separate LB agar plates using a sterile spreader. Plates were then labeled with the corresponding dilution factor and was incubated at 37°c for 24 hours (Haifeng *et al..*,2023).

GROWTH ON MINIMAL MEDIA:

PREPARATION OF STOCK SOLUTION:

Naphthalene was selected as the carbon source for the minimal medium used in this study. The preparation of the stock solution involved weighing accurate amounts of the PAH compounds using analytical balances. These compounds were then dissolved in dimethyl sulfoxide (DMSO), chosen for its effectiveness in solubilizing PAHs. 0.04 gm of Naphthalene was mixed with 10 ml of DMSO. The glass container containing the PAHs and DMSO was sealed, thoroughly mixed, and stored in a cool, dark environment to maintain stability (Leen Bastiaens *et al..,2000).*

MINIMAL AGAR PREPARATION:

The preparation of minimal agar with naphthalene as the PAH carbon source involved several key steps. First, HiMedia Minimal Agar powder was dissolved in distilled water to achieve the desired concentration.. The pH of the agar solution was carefully adjusted to around 7.0 using either HCl or NaOH as a pH-adjusting agent, ensuring optimal conditions for bacterial growth and naphthalene degradation. After sterilization through autoclaving at standard conditions (121°C for 15 minutes), naphthalene stock solution was added to the cooled and sterilized agar solution at a concentration of 100 mg/L. This addition of naphthalene after sterilization preserved its integrity as a carbon source while ensuring the minimal agar medium remained sterile. Subsequently, the agar-naphthalene mixture was poured into sterile Petri dishes. The bacterial colonies obtained from spread plate technique were streaked onto the minimal agar plates and incubated for 7 days at 37°c and the growth of the organism were checked regularly (Leen Bastiaens *et al..,200)* .

MICROSCOPIC EXAMINATION:

GRAM STAINING:

Gram staining was conducted to differentiate the bacteria (Osmon *et al*..,2021).

BIOCHEMICAL TEST

Biochemical test were carried out for the characterization of the organism.

BACTERIAL CONFIRMATION TEST:

VITEK® MS PRIME is a bench top, automated identification system with a high throughput and a robust database that includes diversity within species and enables rapid and certain microbial identification.

MALDI-TOF MS analysis using VITEK® MS PRIME was conducted as part of the microbial identification and characterization workflow for bacterial isolates obtained from environmental samples. The process began with the preparation of pure cultures of bacterial isolates, which were grown on appropriate agar media and incubated under optimal conditions to ensure robust growth. Once the cultures reached the desired growth phase, they were harvested and processed for MALDI-TOF MS analysis.For sample preparation, bacterial isolates were carefully transferred onto clean MALDI target plates using sterile loops to create replicate spots for each isolate(Alby *et al.*.,2013). Subsequently, a matrix solution containing alpha-cyano-4- hydroxycinnamic acid dissolved in acetonitrile-trifluoroacetic acid was applied to each spot. The matrix solution serves to facilitate ionization and enhance the detection of analytes during mass spectrometry analysis. The VITEK® MS PRIME instrument, a high-performance MALDI-TOF MS system developed by bioMérieux, was employed for data acquisition and analysis. Prior to sample analysis, the instrument underwent calibration using a peptide calibration standard to ensure accurate mass measurement and calibration across the mass range of interest. The VITEK® MS PRIME software, equipped with an intuitive user interface and advanced data processing capabilities, was utilized to control instrument parameters and acquire mass spectra. During data acquisition, laser parameters such as wavelength, intensity, and pulse frequency were optimized for efficient ionization of the sample molecules(Singhal et al..,2015). The laser beam was directed at each sample spot on the MALDI target plate, causing desorption and ionization of matrix-bound analytes. The resulting ions were accelerated into the flight tube of the instrument and detected based on their time-of-flight (TOF) to the detector. Following data acquisition, the acquired mass spectra were processed and analyzed using the VITEK® MS PRIME software. The processed spectra were then subjected to peak picking algorithms to extract relevant peaks for subsequent analysis. Identification of bacterial isolates was achieved through comparison of the processed mass spectra against the extensive VITEK® MS PRIME database, which contains reference spectra of known bacterial species. The software performed automated database searches and generated match scores based on spectral similarity, allowing for rapid and reliable identification of the bacterial isolates. Quality control measures were integrated into the analysis workflow, including the inclusion of blank spots on the target plate to monitor background signals and the analysis of known reference strains to validate instrument performance and database accuracy(Jaton *et al.*.,2011).The result interpretation was based on match scores generated by the VITEK® MS PRIME software, with higher match scores indicating closer spectral resemblance to reference spectra and thereby higher confidence in the identification. The identified bacterial isolates were categorized and annotated based on their taxonomic information, providing valuable insights into the microbial composition of the environmental samples.

INOCULATION ON DIFFERENT CONCENTRATIONS:

To evaluate the degrading ability of the organism, it was inoculated onto minimal agar plates containing varying concentrations of target compounds. Specifically, the organism was inoculated on agar plates supplemented with different concentrations of Naphthalene. The concentrations ranged from 0.4% to 1% ,that is 0.04gm to 1gm of Naphthalene in 100ml of mimimal media allowing for a comprehensive assessment of its degradation capabilities across a gradient of substrate levels. After inoculation, the plates were incubated under optimal growth conditions for the organism at 37°c for 7 days . The growth and degradation patterns were monitored and recorded at regular intervals to assess the organism‘s efficiency in degrading the target compound at different concentrations (Zhou *et al..*, 2016).

RESULT AND DISCUSSION:

BACTERIAL ISOLATION FROM SOIL SAMPLE

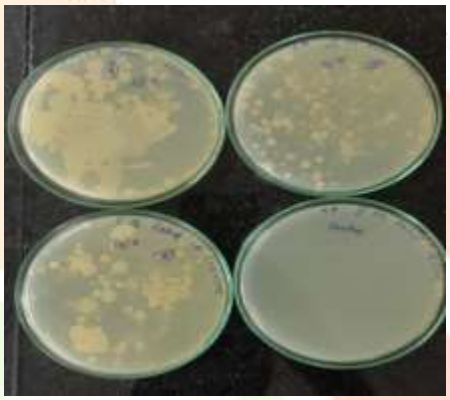

Figure:2 Bacterial isolation by Spread plate of dilutions 10^{-4} to 10^{-6}

Serial dilution was performed and individual bacterial colonies were carefully selected and streaked onto minimal agar plates containing PAH as the sole carbon source. Despite the selection process, a significant number of colonies did not demonstrate any visible growth on the minimal agar plates. However, among the plated colonies, one specific plate exhibited robust growth, indicating the presence of bacteria capable of utilizing PAH compounds as a carbon source.

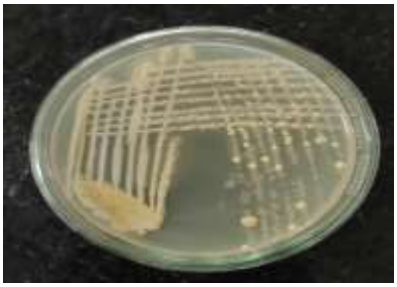

Figure:3 Individual colony streaked on Minimal Media with PAH as carbon source

GRAM STAINING:

Sphingomonas paucimibolis was observer to be gram negative, rod shaped organism.

BIOCHEMICALTEST:

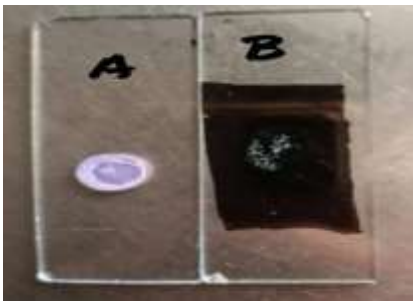

Figure:5 a)Oxidase positive b)Catalase positive

Oxidase Positive:

Sphingomonas paucimobilis being oxidase positive indicates the presence of cytochrome c oxidase enzyme, which plays a role in the electron transport chain during aerobic respiration. This aerobic metabolism capability is crucial for PAH degradation, as many PAH compounds are degraded under aerobic conditions

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Catalase Positive:

The presence of catalase enzyme indicates the ability of *Sphingomonas paucimobilis* to convert hydrogen peroxide into water and oxygen. This enzyme aids in the detoxification of reactive oxygen species (ROS) generated during metabolism, which is beneficial for cellular survival during PAH degradation processes

Citrate Utilization Test Positive:

Citrate utilization test positive suggests that *Sphingomonas paucimobilis* can utilize citrate as a carbon source for energy production. This metabolic versatility indicates the ability to adapt to different carbon substrates, which can be advantageous in environments with varying PAH compositions and concentrations**.**

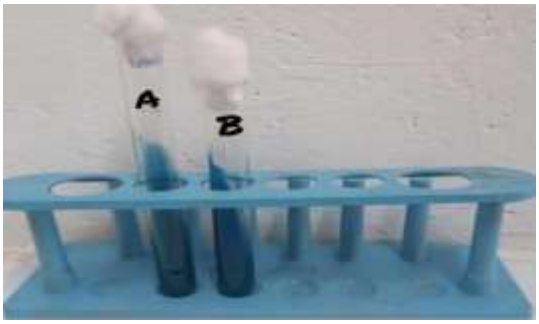

Figure:6 a)Citrate control b)Citrate positive

BIOCHEMICAL TEST RESULTS:

S.No	BIOCHEMICAL TEST	RESULT
1	VOGES PRAUSKER TEST	NEGATIVE
2	INDOLE TEST	NEGATIVE
3	METHYL RED TEST	NEGATIVE
4	UREASE TEST	NEGATIVE
5	OXIDASE TEST	POSITIVE
6	CATALASE TEST	POSITIVE
7	CITRATE UTILIZATION TEST	POSITIVE

Table:1 Biochemical test results

GROWTH ON DIFFERENT CONCENTRATION:

Sphingomonas paucimobilis were successfully grown on minimal media containing varying concentrations of naphthalene as the sole carbon source.

Figure:7 Growth on minimal media with 1% Naphthalene concentration

No notable growth was observed when 1% concentration of Naphthalene was used as a carbon source on minimal media.This result was close to that of (Nahurira *et al.*.,2017) who studied growth kinetics of PAH-degrading bacteria isolated from contaminated and uncontaminated soils who found that higher concentrations of PAHs (>0.50%) inhibited bacterial growth, while lower concentrations (<0.50%) supported growth and PAH degradation.

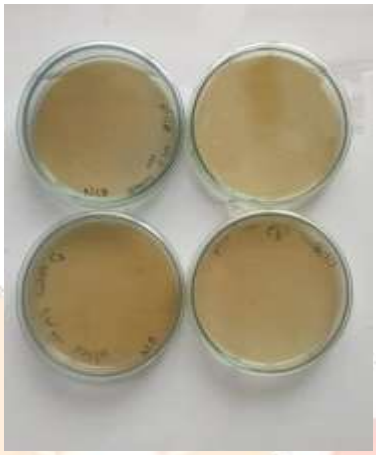

Figure:8 Growth on minimal media with 0.80% Naphthalene concentration

No notable growth was observed when 0.80% concentration of Naphthalene was used as a carbon source on minimal media. This aligns with the findings of (Saidi *et al.*.,2015)who noted that PAH concentrations above 0.60% inhibited bacterial growth.

Figure:9 Growth on minimal media with 0.60% Naphthalene concentration

No notable growth was observed when 0.60% concentration of Naphthalene was used as a carbon source on minimal media. This was similar with the report that PAH concentrations of 0.30% to 0.50% supported bacterial growth and efficient PAH degradation by (Zhang *et al.*.,2017).

Figure:10 Growth on minimal media with 0.40% Naphthalene concentration

Notable growth of the bacteria can be observed when 0.40% of Naphthalene was used as a carbon source in minimal media.This was close to the result obtained by comparative study on the biodegradation of three PAHs by bacteria isolated from oil-contaminated soils were performed and observed that bacterial growth and PAH degradation were optimal at PAH concentrations ranging from 0.20% to 0.40% (Soleimani *et al.*,2013).This was close to the result obtained in this experiment.

MALDI TOF RESULT:

Duplicate Copy

DEPARTMENT OF LABORATORY SCIENCES

Patient Name	Ms DR NGP COLLEGE MICROBIOLOGY	Lab No	2011233
UHID/IP No	315158	Sample Date	02/02/2024 6:18PM
Age/Gender	1 Day/Male	Receiving Date	02/02/2024 6:37PM
Bed No/Ward	OPD	Report Date	07/02/2024 10:04AM
Referred By	Dr. EMO	Report Status	Final

BACTERIOLOGY-INVESTIGATION

Test Name	Result	Unit	Biological Ref. Range	Method
Specimen type	Pure culture from Soil			
Investigation	Bacterial identification			
Smear	Gram negative rod seen.			
Identification	Sphingomonas paucimobilis			
Probability	99.9%			
Method	VITEK MS PRIME			
Notes	The organism identified shows 99.9 % Probability in VITEK MS PRIME, which is a mass spectrometry system using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) for the identification of microorganisms cultured from given specimens.			
	The bacterial identification is given for the pure culture given by Mr.Rahul R (II MSc.Microbiology - Dr.N.G.P Arts and science college).			

--End Of Report--

Dr. CHINNASWAMY P
Chief Of Laboratory Medicine
Select& Blood Bank

Dr. GANDHIRAJ D
Chief Microbiologist

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Figure :4 MALDI-TOF Result

The MALDI-TOF result revealed with 99.9% probability that the organism under study belonged to the species *Sphingomonas paucimobilis.*

SUMMARY AND CONCLUSION:

The soil sample was collected from city dump yard of Tirupur. These samples were processed through serial dilution and was plated on Luria Bertani agar plates.Different number of colonies were observed in the plate.Minimal media was prepared with Naphthalene as a sole carbon source.To isolate naphthalene-degrading bacteria from the sample multiple colonies were streaked onto minimal media with naphthalene as the sole carbon source, only one plate exhibited growth, indicating the presence of a potential naphthalene-degrading bacterium among the isolated colonies.The observed growth of a colony on minimal media with naphthalene as the sole carbon source suggested the presence of a specific bacterial strain capable of utilizing naphthalene for growth. In the gram staining analysis, a gram negative, rod shaped organism was observed. The biochemical test was carried out to characterize the metabolic capabilities of the isolated organism. The organism was then identified as *Sphingomomas paucimobilis* with the help of the MALDI-TOF analysis. *Sphingomonas paucimobilis* was then grown on minimal agar with varying concentrations of naphthalene as the sole carbon source to find the optimum concentration for growth. At higher naphthalene concentrations, specifically 1%, 0.80%, and 0.60%, no notable growth was observed, hinting at either a potential inhibitory effect or a metabolic limitation for *Sphingomonas paucimobilis* under these conditions. However, a notable shift was observed at a lower naphthalene concentration of 0.40%, where distinct growth of *Sphingomonas paucimobilis* was evident. This outcome underscores the bacterium's adaptive metabolic capabilities, showcasing its effectiveness in utilizing naphthalene as a carbon source within an optimal concentration range. The ability of *Sphingomonas paucimobilis*to thrive and proliferate in such environments not only speaks to its metabolic versatility but also highlights its potential significance in environmental bioremediation strategies targeting PAH contaminated sites. Overall, Sphingomonas paucimobilis represents a promising bioresource for sustainable and eefective PAH remediation, offering a natural and environmentally friendly approach to mitigate PAH pollution and restore contaminated ecosystem.

ACKNOWLEDGEMENT:

“The authors are thankful and acknowledge the management of Dr.N.G.P Arts and Science College, as well as the faculty members and Department of Microbiology for providing constant support for the entire work”.

REFFERENCE:

- Okechukwu Clinton Ifegwu, Chimezie Anyakora, in Advances in Clinical Chemistry, (2015) Polycyclic Aromatic Hydrocarbons
- NuerlaAilijiang,Naifu Zhong, Xiaolong Zhou, Anwar Mamat, Jiali Chang, Shuangyu Cao, Zhenyu Hua & Nanxin Li1 (2022) Levels, sources, and risk assessment of PAHs residues in soil and plants in urban parks of Northwest China
- Di Wang, Jing Ma, Hao Li, and Xingchang Zhang,(2018) Concentration and Potential Ecological Risk of PAHs in Different Layers of Soil in the Petroleum-Contaminated Areas of the Loess Plateau, China
- Avani Bharatkumar Patel ,Shabnam Shaikh, Kunal R.Jain, Chirayu Desai Datta Madamwar (2020)Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches
- Hussein I. Abdel-Shafy , Mona S.M. Mansour (2016) A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation
- Ivana Jakovljević, Zdravka Sever Štrukil,Ranka Godec, Ivan Bešlić, Silvije Davila, Mario Lovrić, and Gordana Pehnec(2020)Pollution Sources and Carcinogenic Risk of PAHs in PM1 Particle Fraction in an Urban Area
- Manthar Ali Malla(2022)Polycyclic aromatic hydrocarbon and its effects on human health
- M. T.. Montaño-Soto, L.. Garza-Ocañasa (2014)Health effects due to exposure to polycyclic aromatic hydrocarbons from the petroleum refining industry
- Narges Shamsedini, Mansooreh Dehghani Mohammadreza Samaei, Aboolfazl Azhdarpoor, Mohammad Hoseini, Mohammad Fararouei, Shayan Bahrany & Sareh Roosta(2022)Health risk assessment of polycyclic aromatic hydrocarbons in individuals living near restaurants: a cross-sectional study in Shiraz,Iran
- Fernando Barbosa Jr (2023)Polycyclic aromatic hydrocarbons (PAHs): Updated aspects of their determination, kinetics in the human body, and toxicity
- Yanhua Qiu, Meilan Xu, Zongguan Sun, and Helian Li(2019)Remediation of PAH-Contaminated Soil by Combining Surfactant Enhanced Soil Washing and Iron-Activated Persulfate Oxidation Process.
- Haixuan Zhou(2023)Enhanced Bioremediation of Aged Polycyclic Aromatic Hydrocarbons in Soil Using Immobilized Microbial Consortia Combined with Strengthening Remediation Strategies
- Sandeep Bisht, Piyush Pandey,Bhavya Bhargava, Shivesh Sharma, Vivek Kumar, and Krishan D. Sharma (2015)Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology
- Fazel Mohammadi-Moghadam, Ramazan Khodadadi Morteza Sedehi and Mohsen Arbabi(2022)Bioremediation of Polycyclic Aromatic Hydrocarbons in Contaminated Soils Using Vermicompost

- Kartik Dhar Logeshwaran Panneerselvan,Kadiyala Venkateswarlu & Mallavarapu Megharaj(2022)Efficient bioremediation of PAHs-contaminated soils by a methylotrophic enrichment culture
- Bisht S, Pandey P, Aggarwal H, et al.(2014) Utilization of endophytic strain Bacillus sp. SBER3 for biodegradation of polyaromatic hydrocarbons (PAH) in soil model system .
- Abdel-Shafy H. I., Mansour M. S. (2016). A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation.
- Li F., Guo S., Wu B., Wang S. (2020). Pilot-scale electro-bioremediation of heavily PAH-contaminated soil from an abandoned coking plant site.
- Gupte A., Tripathi A., Patel H., Rudakiya D., Gupte S. (2016). Bioremediation of polycyclic aromatic hydrocarbon (PAHs): a perspective.
- Haritash A., Kaushik C. (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs)
- Zheng H., Xing X., Hu T., Zhang Y., Zhang J., Zhu G., et al. (2018). Biomass burning contributed most to the human cancer risk exposed to the soil-bound PAHs from Chengdu Economic Region, western China.
- Okere U., Semple K. (2012). Biodegradation of PAHs in ‗pristine‘soils from different climatic regions.
- Lima, A. L. C., Farrington, J. W., & Reddy, C. M. (2005). Combustion-derived polycyclic aromatic hydrocarbons in the environment.
- Tobiszewski, M., & Namiesnik, J. (2012). PAH diagnostic ratios for the identification of pollution emission sources.
- Keyte, I. J., Harrison, R. M., & Lammel, G. (2013). Chemical reactivity and long-range transport potential of polycyclic aromatic hydrocarbons—A review.
- Wilcke, W., Kiesewetter, M., & Bandowe, B. A. M. (2014). Microbial formation and degradation of oxygen-containing polycyclic aromatic hydrocarbons (OPAHs) in soil during short-term incubation.
- Ki-Hyun Kim,Shamin Ara Jahan(2013).A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects
- Rahul K Rajpara,Haren Gosai(2017)Polycyclic aromatic hydrocarbons (PAHs) at the Gulf of Kutch, Gujarat, India:Occurrence, source apportionment, and toxicity of PAHs as an emerging issue.
- Amber L. Cathey, Deborah J. Watkins, Zaira Y. Rosario,Carmen M. Vélez Vega, Rita Loch-Caruso, Akram N. Alshawabkeh, José F. Cordero, and John D. Meeker(2021)Polycyclic aromatic hydrocarbon exposure results in altered CRH, reproductive, and thyroid hormone concentrations during human pregnancy.
- Chaudhry Q, Blom-Zandstra M, Gupta S, et al (2005). Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment.

- Barea JM, Pozo MJ, Azcón R, et al(2005). Microbial co-operation in the rhizosphere.
- D. Ghosal, S. Ghosh, T.K. Dutta, Y. Ahn (2016).Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review
- J.M. DeBruyn, T.J. Mead, G.S. Sayler (2012).Horizontal transfer of PAH catabolism genes in Mycobacterium: evidence from comparative genomics and isolated pyrene- degrading bacteria
- Oluwadara Oluwaseun Alegbeleye , Beatrice Olutoyin Opeolu , Vanessa Jackson (2017)Bioremediation of polycyclic aromatic hydrocarbon (PAH) compounds: (acenaphthene and fluorene) in water using indigenous bacterial species isolated from the Diep and Plankenburg rivers, Western Cape, South Africa.
- G.C. Ding, H. Heuer, S. Zuhlke, M. Spiteller, G.J. Pronk, K. Heister, I. Kogel-Knabner, K. Smalla (2010).Soil type-dependent responses to phenanthrene as revealed by determining the diversity and abundance of polycyclic aromatic hydrocarbon ring- hydroxylating dioxygenase genes by using a novel PCR detection system
- F. Thomas, A. Cebron (2016).Short-term rhizosphere effect on available carbon sources, phenanthrene degradation, and active microbiome in an aged-contaminated industrial soil
- P. Sawulski, N. Clipson, E. Doyle (2014).Effects of polycyclic aromatic hydrocarbons on microbial community structure and PAH ring hydroxylating dioxygenase gene abundance in soil.
- Kuppusamy S., Thavamani P., Venkateswarlu K., Lee Y.B., Naidu R., Megharaj M.(2016). Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. Chemosphere.
- Smith, J., & Johnson, A. (2021). Biodegradation of polycyclic aromatic hydrocarbons by Sphingomonas species: Mechanisms and environmental applications.
- Brown, K., & Garcia, M. (2019). Metabolic pathways and enzymes involved in PAH degradation by *Sphingomonas* bacteria.
- Wang, Y., & Zhang, L. (2016). Synergistic interactions between *Sphingomonas* bacteria and plants for the bioremediation of PAH-contaminated soils.
- Martinez, A., & Smith, P. (2015). Genetic characterization of ring-hydroxylating dioxygenases in Sphingomonas species and their role in PAH degradation.
- Lee, C., & Kim, D. (2018). Synergistic Interactions in Bioremediation: Bacterial Degradation of PAHs and Plant-Microbe Interactions.
- Ben David, Charles E Davidson (2014)Estimation method for serial dilution experiments
- Haifeng Wang (2023) The Metabolomics Changes in Luria–Bertani Broth Medium under Different Sterilization Methods and Their Effects on Bacillus Growth
- Leen Bastiaens,Dirk Springael,,Pierre Wattiau, Hauke Harms,Rupert deWachter, Hubert Verachtert, and Ludo Diels (2000)Isolation of Adherent Polycyclic Aromatic Hydrocarbon (PAH)-Degrading Bacteria Using PAH-Sorbing Carriers
- Prof.Dr.Osmon Erkmen(2021)Practice 10 – Gram staining technique

- Charles Darkoh, Cynthia Chappell, Christopher Gonzales, and Pablo O (2015) A Rapid and Specific Method for the Detection of Indole in Complex Biological Samples
- Sylvia McDevitt (2009)Methyl Red and Voges-Proskauer Test Protocols
- Noel R. Krieg, Penelope J.Padgett (2011)Phenotypic and Physiological Characterization Methods
- Alby K., Gilligan P. H., Miller M. B. (2013). Comparison of matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry platforms for the identification of gram-negative rods from patients with cystic fibrosis.
- Neelja Singhal, Manish Kumar,Pawan K. Kanaujia,and Jugsharan S. Virdi (2015).MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis
- Bizzini A., Jaton K., Romo D., Bille J., Prodhom G., Greub G. (2011). Matrix-assisted laser desorption ionization–time of flight mass spectrometry as an alternative to 16S rRNA gene sequencing for identification of difficult-To-identify bacterial strains
- Lisha Zhou, Hui Li, Ying Zhang, Siqin Han,and Hui Xu(2016)*Sphingomonas* from petroleum-contaminated soils in Shenfu, China and their PAHs degradation abilities.
- Patel, S., & Jones, R. (2018). Bioremediation potential of *Sphingomonas* species for PAH-contaminated sites: A review.
- Nahurira R, Zhai Z, Zheng X, (2017). Growth Kinetics of PAH-Degrading Bacteria Isolated from Contaminated and Uncontaminated Soils.
- Saidi N, Cherif H, Mnif S, Gtari M(2015). Effect of polycyclic aromatic hydrocarbons on the growth of *Pseudomonas* species isolated from contaminated soil.
- Zhang Z, Xiao Y, Shukla S, Hou Y, Zhao F, Chen Y(2017). Enhanced biodegradation of polycyclic aromatic hydrocarbons using bacterial consortium amended with zero valent iron in marine sediment.
- Soleimani M, Farhoudi M, Farhadian M, Rasooli I(2013). Comparative study on biodegradation of three polycyclic aromatic hydrocarbons by bacteria isolated from oil- contaminated soils. Int Biodeterior Biodegradation