Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 23 | No. 2 | pp. 785-795 | 2024

Original Research Paper

https://doi.org/10.46488/NEPT.2024.v23i02.015

Open Access Journal

PAHs Biodegradation by Locally Isolated *Phanerochaete chrysosporium* and *Penicillium citrinum* from Liquid and Spiked Soil

Kiran Bishnoi*(**)†, Pushpa Rani*, Minakshi Karwal* and Narsi R. Bishnoi***

*Department of Environmental Science and Engineering, Guru Jambheshwar University of Science & Technology, Hisar-125001, Haryana, India

**Department of Environmental Studies, Govt. College for Women, Hisar-125001, Haryana, India

***Department of Applied Sciences, KIET Group of Institutions, Delhi-NCR, Ghaziabad-201206, U.P., India

†Corresponding author: K. Bishnoi; kiranbishnoi@gmail.com

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 23-08-2023
Revised: 29-10-2023
Accepted: 01-11-2023

Key Words:
Biodegradation
Phenanthrene
Polycyclic aromatic hydrocarbons
Phanerochaete chrysosporium
Penicillium citrinum

ABSTRACT

In the present study, biodegradation of polycyclic aromatic hydrocarbons (PAHs) was examined using two fungal strains, namely P. chrysosporium and P. citrinum, isolated from locally contaminated soil. These two fungal strains were compared based on degradation properties under standardized conditions (pH 7.0, temperature 30°C, carbon source yeast extract) using PAH sole and a mixture of five different PAHs. In liquid media, PAH degradation was higher as compared to spiked soil by *P. chrysosporium*, followed by *P. citrinum*. In liquid culture, maximum degradation was 96.13% phenanathrene, 86.34% fluoranthene, 72.75% pyrene, 52.25% chrysene, and 40.16% benzo(a)pyrene by *P. chrysosporium*. PAH degradation in spiked soil was 78.5% phenanthrene, 65.91% fluoranthene, 61.73% pyrene, 48.2% chrysene, and 26.82% benzo(a)pyrene within 28 days by *P. chrysosporium*. Both local fungal isolates showed potential for degradation of PAHs alone and in PAH mixtures.

INTRODUCTION

Unorganised and unmanaged expansion activities pose a severe threat to the environment by introducing harmful chemicals/ substances into the environment like polycyclic aromatic hydrocarbons (PAHs). PAHs are a large group of organic compounds having two or more fused aromatic rings, mainly having C and H atoms (Jacques et al. 2008, Bright et al. 2023). More than 500 different PAHs are present in the environment, but US EPA listed 16 PAHs under the ''priority pollutants'' category (US EPA 2002), many of which are well-known as mutagens, carcinogens, and teratogens (Abdel-Shafy & Mansour 2016). Both natural and anthropogenic sources are responsible for their wide distribution in the environment. Due to their bioaccumulation tendency, they enter the food chain. Natural sources include fires (forest and grassland), volcanic eruptions, oil seeps, exudates from trees, etc. (Babu et al. 2019). Anthropogenic sources include residential heating, burning (coal and wood), coal gasification and liquefying plants, carbon black, coal-tar pitch and asphalt production, coke and aluminum production, petroleum refineries, vehicular emissions and incineration of municipal waste (Abdel-Shafy & Mansour 2016, Alekseev & Abakumov 2021). PAHs are widely dispersed at various levels in soils, river water and sediments, groundwater, and atmosphere (Bishnoi et al. 2009, Malik et al. 2010, Masih et al. 2010, Gupta & Kumar 2020). Some PAHs have also been found in fruits and vegetables in trace amounts, which may have been transferred from air and soil during cultivation or transportation and storage (Bishnoi et al. 2006, Paris et al. 2018).

Due to their boundless occurrence and health effects, it has become a need of the hour to carry out remediation of PAHs. Different types of physical, chemical, and biological methods can be used for PAH remediation. While PAHs may undergo adsorption, chemical oxidation, photolysis, and volatilization, neutralization by microbial activities is the primary process affecting the persistence of PAHs in contaminated sites (Ghosal et al. 2016, Thacharodi et al. 2023). Bioremediation of PAHs is considered an efficient, economical, versatile, and ecologically acceptable treatment. Both fungi and bacteria can metabolize different types of PAHs, but their pathways for degradation are different. Many recent studies reported several bacteria and filamentous fungal species having the capability for

mineralization or degradation of PAHs (Liu et al. 2017, Gu et al. 2021). Bioremediation using fungi-based technology, renowned as mycoremediation, is gaining more attention for the degradation of PAHs. Different fungal strains such as *Phanerochaete chrysosporium, Trichoderma harzianum, Pleuroyus ostreatus, Trametes versicolor, Cunninghamella elegans, Aspergillus niger* and *Penicillium janthinellum* can degrade a variety of PAHs to polar compounds (Kadri et al. 2017, Al-Hawash et al. 2018, Etim et al. 2022). Most of the studies on fungal strains for PAH degradation are divided into ligninolytic and non-ligninolytic fungi. Improving this microbial-based bioremediation technique includes the exploration of new strains capable of wide spectrum degradation of PAHs and optimization of influencing factors affecting degradation rate (Singh & Tiwary 2017, Ibrahim et al. 2018).

The degradation of individual PAHs by pure and mixed microbial communities has been studied by several researchers (Zhong et al. 2011, Sonwani et al. 2019). However, contaminated sites are generally occupied with complex mixtures of PAHs, and the degradation rate and extent of degradation for individual PAHs vary depending on the presence of other hydrocarbons (Sawulski et al. 2015). To be implemented as a remediation technology, mycoremediation has to degrade a mixture of PAHs from the contaminated site.

Phenanthrene was chosen as a model compound for studying the degradation process, as it is a frequently occurring and abundant PAH pollutant. The present study aims at screening the fungal strains isolated from PAH-contaminated sites and optimizing the degradation of some frequently occurring and abundant PAHs with the isolated strains.

MATERIALS AND METHODS

Culture Medium

A basal salt media (BSM) (Saraswathy & Hallberg 2002) along with a 0.01% mixture of five PAHs (phenanthrene, acenaphthene, anthracene, fluoranthene, and pyrene) as carbon source, were used for enrichment. Rose Bengal Agar (RBA) Medium ($g.L^{-1}$): dextrose, 10; peptone, 5; $MgSO_4.7H_2O$, 0.5; K_2HPO_4, 1.0; Rose Bengal, 0.033; and streptomycin, 0.033 and agar 2% was used for fungus growth on Petri plates. Potato Dextrose Agar (PDA) slants were used for maintaining fungal strains at 4°C.

Isolation of PAHs Degrading Fungal Strains

Fifty soil samples were collected from different contaminated sites like highways, refineries, and sewage-irrigated soil from Panipat, Hisar, and Faridabad cities of Haryana, India, for isolation of fungal strains. The soil sample (10 g) was mixed with deionized water (100 mL) and placed in a shaker (120 rpm) overnight at 28°C in 250 mL Erlenmeyer flasks. After that, the supernatant (5 mL) was added to BSM plus trace element solution (45 mL) in 250 mL Erlenmeyer flasks amended with 0.01 % phenanthrene and was shaken for five days at 28°C and 120 rpm in shaker cum-incubator. The inoculum (10%) from previous experiments was taken and subcultured in the same medium for the enrichment process. The enrichment action was repeated for 3 months and the next three months in a 0.01% mixture of five PAHs (phenanthrene, acenaphthene, anthracene, fluoranthene, and pyrene). The 0.1 mL culture of 10-fold diluted PAHs enriched culture was spread on RBA plates supplemented with streptomycin ($0.033\ g.L^{-1}$) and incubated for 5 days at 28°C. The fungal colonies grown on plates were purified by repeated striking methods. A total of 23 fungal strains were isolated. Among all the isolates, 10 fungal strains were selected for further study, deepening upon their growth ability on agar medium containing a mixture of five PAHs.

Preparation of Fungal Inocula

Fungal inocula used for degradation experiments were prepared by growing the respective fungal isolate on PDA slants for 7-10 days. Following sufficient growth, 10 mL of sterilized distilled water was pipetted into a slant and gently agitated to suspend the spores. The spore suspension was then used to inoculate 100 mL sterile BSM media in a 250 mL conical flask incubated for 48 hours at 30°C at 120 rpm. After 48 hours of incubation in an incubator shaker, the mycelial pellets formed were filtered through Whatman #1 filter paper and washed twice using sterilized distilled water. Before inoculation, mycelium was homogenized with the help of a magnetic stirrer. 1 mL of homogenized mycelial suspensions ($1.0\ g.L^{-1}$ wet biomass) was used as inoculum for further study.

Screening of Fungal Strains

Fungal strains were screened for the selection of efficient fungus based on oven-dry weight at 60°C and percent degradation of PAH in BSM media after 7 days of incubation time. Out of 10, two fungal strains were screened, namely *Phanerochaete chrysosporium* (isolated from Panipat refinery soil) and *Penicillium citrinum* (isolated from sewage irrigated soil contaminated with PAHs, Hisar) identified from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh and Indian Agricultural Research Institute (IARI), New Delhi), respectively, based on their best performance.

Experimental Design for Optimization Study

Experiments were carried out in 25 mL Erlenmeyer flasks

This publication is licensed under a Creative Commons Attribution 4.0 International License

having 9 mL of sterilized (autoclaving at 15 psi and 121°C for 20 min) Basal Salt medium plus 1mL mycelial suspension (1.0 g.L^{-1} wet biomass) pre-culture fungal inoculums agitated at 120 rpm in BOD cum incubator shaker. Culture conditions and standardization of various parameters (pH, temperature, phenanthrene concentration, and time) were studied. Based on the screening experiment, selected fungal strains were further studied at different pH (5.0, 6.0, 7.0, 8.0, and 9.0), temperature (20°C, 30°C and 40°C), phenanthrene concentration (50, 100, 150, and 200 ppm) and time (7, 14, 21 and 28 days). To see the effect of carbon sources on the degradation of PAH under optimized conditions, three different carbon sources were used (yeast extract, peptone, and glucose at 5 g.L^{-1}).

Biodegradation Study of PAHs Mixture

Liquid medium: Biodegradation experiments were carried out in 25 mL Erlenmeyer flasks having 9.0 mL sterilized basal salts medium with 5 g.L^{-1} yeast extract (BSMY) and 1 mL of homogenized mycelial suspensions (1.0 g.L^{-1} wet biomass) containing combination of five different PAH (200 ppm phenanthrene, 100 ppm of fluoranthene and pyrene, and 50 ppm of chrysene and benzo(a)pyrene).

Spiked soil: Bioremediation experiments (in triplicate) were performed in Erlenmeyer flasks (100 mL) having 10 g of sterilized agricultural soil (unexposed to PAHs) samples. The soil was spiked with a solution containing a range of PAHs in hexane to give a final soil concentration of 100 mg.kg^{-1} for fluoranthene and pyrene, 200 mg.kg^{-1} for phenanthrene, and 50 mg.kg^{-1} for chrysene and benzo(a)pyrene. The spiked soil was placed in a fume hood for 2 hours for evaporation of hexane. 9 mL BSMY medium containing 1 mL homogenized mycelial suspensions (1.0 g.L^{-1} wet biomass) added in 10 g spiked soil. Microcosms were incubated at 30°C with pH 7.0 in the dark for 28 days at 70% humidity in the humidity cabinet.

All three flasks were analyzed at 0, 7, 14, 21 and 28 days. Results were expressed as means. The standard deviation was less than 2% of the mean.

Quantification of PAHs

PAH extraction of both samples (liquid and soil) were carried out by adding hexane (20 mL) to each flask, which was kept in a BOD cum incubator shaker at 4°C and 120 rpm for 4 h. After that, the samples were placed in an ultrasonicator (Trans-sonic) for 15 min for two times for proper separation of the organic phase from the aqueous phase. The organic phase sample was cleaned up using chromatography to remove impurities and, after that, concentrated on the rotary evaporator (JSGW) two times with hexane. After that, the sample was centrifuged for 10 min at 10000 rpm and filtrated with syringe filters (0.45µm). High-performance liquid chromatography (HPLC Water 600) equipped with UV-detector and C-18 column was used for the analysis of samples. The mobile phase used was an acetonitrile-water mixture (75:25) with a maintained flow rate of 1.0 mL min^{-1}. The sample (20 µL) was injected into the column through a sample loop. The concentration of PAH was calculated by comparing peak areas of the sample chromatogram and standard chromatogram (Bishnoi et al. 2008).

The Concentration of PAH (ppm):

$$= \frac{\text{Peak Area of Chromatogram of the Sample}}{\text{Peak Area of Chromatogram of Standard PAH Compound}} \times \text{Concentration of Standard PAH Compound}$$

Biodegradation efficiency was calculated as:

$$\text{Biodegradation efficiency (\%)} = \frac{(C_0 - C_e)}{C_0} \times 100$$

Where C_0 is the initial concentration of PAHs (mg.kg^{-1}); Ce is the residual concentration of PAHs (mg.kg^{-1}).

RESULTS AND DISCUSSION

Isolation and Screening

Among 23 fungal isolates, 10 (coded F1, F2, F3...F10) were selected for further screening in BSM medium with 50 ppm phenanthrene concentration after 7 days incubation period. Independent of fungi species, studied for phenanthrene degradation and mycelial growth to check their best performance. Enzymatic activities were also studied for the selection of fungal strains (data not shown). Among 10 fungal strains, the maximum degradation of phenanthrene was 17.56% and 10.23% with *P. chrysosporium* (F2) and *P. citrinum* (F5), respectively (Fig. 1). The highest mycelial growth was also obtained with these two isolates 5.73 g.L^{-1} and 6.42 g.L^{-1}. Maximum degradation of phenanthrene was found with *P. chrysosporium* fungal strain. Still, dry-weight fungal biomass was found to be maximized in the *P. citrinum* strain (Fig. 1). Some other fungal strains showed high dry-weight biomass but less percent degradation. This showed that there is no direct correlation between percent degradation and biomass weight. Quintero et al. (2008) observed a similar effect with the degradation of hexachlorocyclohexane (HCH) isomers on white rot fungi. In this study, the mycelial growth of *P. chrysosporium* was higher than *Bjerkandera adusta* in the presence of HCH isomers, but the degradation rate was high with *B. adusta*.

Effect of Environmental Factors on Phenanthrene Degradation

Environmental factors like pH, temperature, initial dose

This publication is licensed under a Creative Commons Attribution 4.0 International License

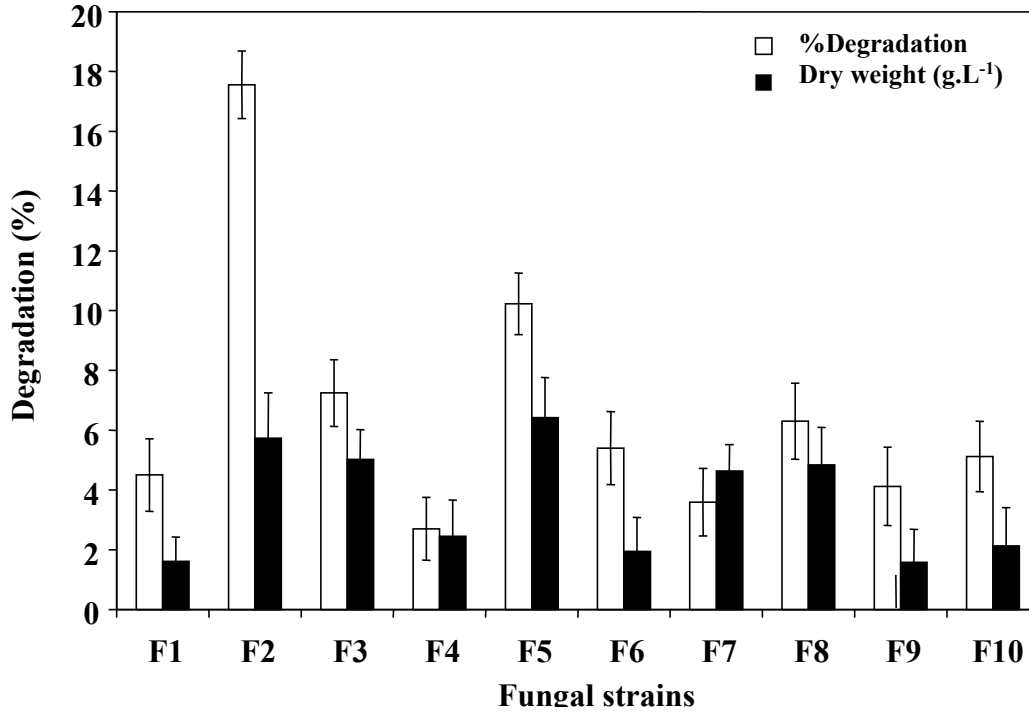

Fig. 1: Comparison of percent degradation and dry weight during degradation of phenanthrene with 10 isolates.

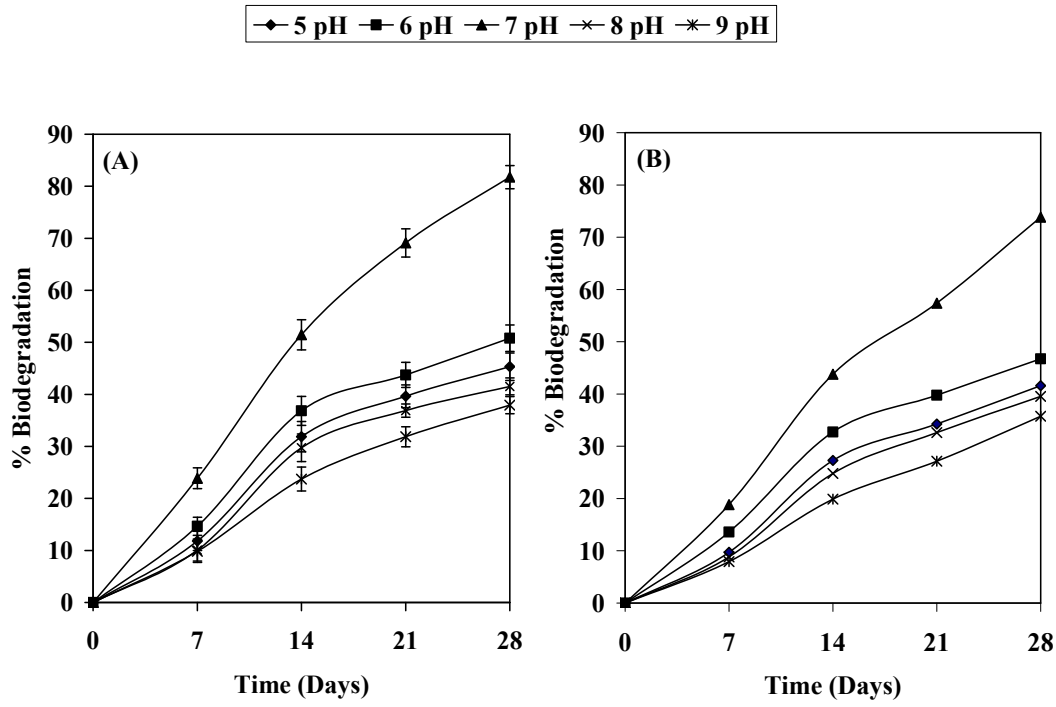

Fig. 2: Effect of different pH values on biodegradation of phenanthrene by (A) *P. chrysosporium* and (B) *P. citrinum* (50 ppm).

This publication is licensed under a Creative Commons Attribution 4.0 International License

of PAHs, type of fungal strain, etc., play a critical role in the degradation of these recalcitrant toxic compounds (Shankhwar & Paliwal 2021). A series of experiments were conducted with both the fungal strains to test phenanthrene degradation at various pHs from 5.0 to 9.0 and temperatures ranging from 20°C to 40°C. The phenanthrene degradation increased with an increase in pH from 5.0 to 7.0 and again decreased with a further increase in pH from 7.0 to 9.0. Maximum degradation of PAH was observed at pH 7.0 by both fungal strains (Fig. 2). The optimal temperature for degradation of phenanthrene was observed at 30°C (Fig. 3), whereas biodegradation efficiency decreased as temperature increased or decreased. Temperature is a crucial factor in the bioavailability of PAH molecules (Okere & Semple 2012). The increased temperature decreased oxygen solubility, which led to a reduction in the metabolic activity of aerobic microbes. Optimal conditions for both the fungal strains were determined to be 30°C and pH 7.0. Microbial activity was slower with an increase or decrease in temperature up to its optimal value. It was also found that the phenanthrene concentration was 78.54 % and 74.8 % with *P. chrysosporium* and *P. citrinum*, respectively, at 50 ppm concentration, but at 200 ppm concentration, the rate of degradation was low (Fig. 4), which slightly differs from the report of Ting et al. (2011). The higher concentration of PAHs results in a reduced degradation rate due to the increased toxicity of PAH metabolites, and microbial community growth is affected due to an increase in toxicity (Bishnoi et al. 2008, Qi et al. 2017). Adverse environmental conditions (like pH and temperature) may reduce the biodegradation by microbes (Wang et al. 2016).

Effects of Carbon Sources on the Degradation of Phenanthrene

The carbon source is required for microbial growth, and due to the toxicity of PAHs, additional carbon sources must be provided. Fungi can co-metabolize different PAHs in the presence of carbon sources (Shankhwar & Paliwal 2021). In the present study, three carbon sources- yeast extract, glucose, and peptone were compared for their effect on microbial growth (Fig. 5). Phenanthrene degradation was 44.82%, 79.79%, 94.44%, and 100% with *P. chrysosporium* (Fig. 5 A) and 36.48%, 67.41 %,87.24% and 94.82% with *P. citrinum* (Fig. 5 B) at 7, 14, 21 and 28 days, respectively, in presence of yeast extract (5 $g.L^{-1}$). The yeast extract supported more degradation as compared to other carbon sources studied as shown in Fig. 5. A similar finding was observed by Mineki et al. (2015) using yeast extract for the transformation of PAHs in soil. They also noticed similar outcomes when additional carbon substrates, such as lactose, sucrose, and yeast extract,

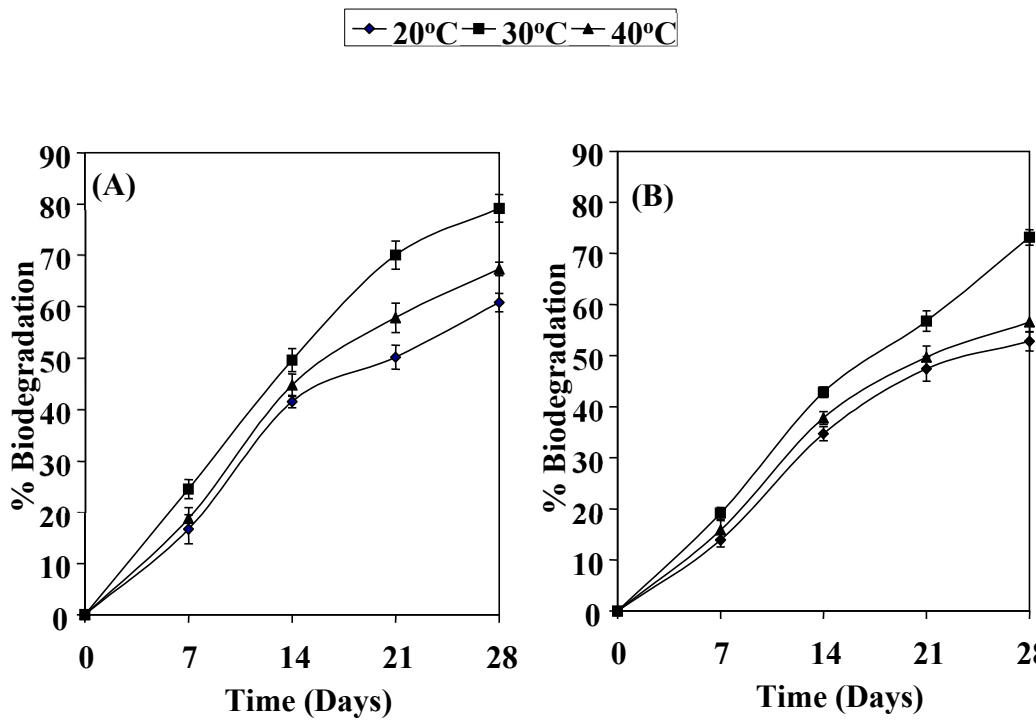

Fig. 3: Effect of different temperatures on biodegradation of phenanthrene by (A) *P. chrysosporium* and (B) *P. citrinum* (50 ppm).

This publication is licensed under a Creative Commons Attribution 4.0 International License

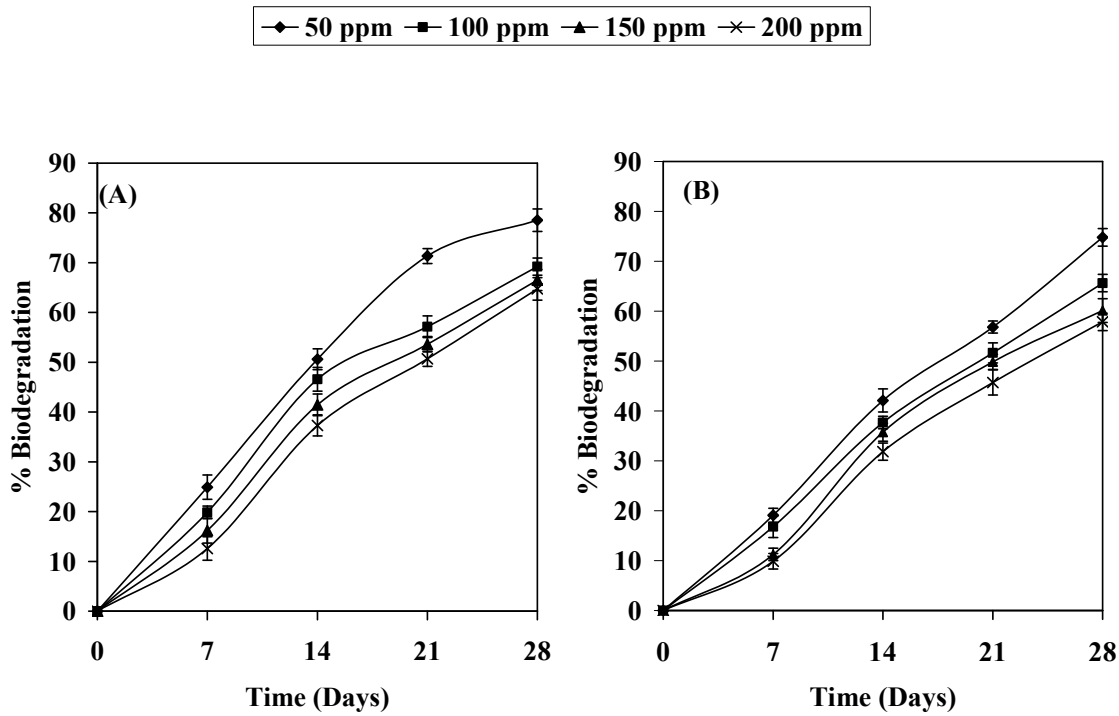

Fig. 4: Effect of different concentrations of phenanthrene on biodegradation of phenanthrene by (A) *P. chrysosporium* and (B) *P. citrinum*.

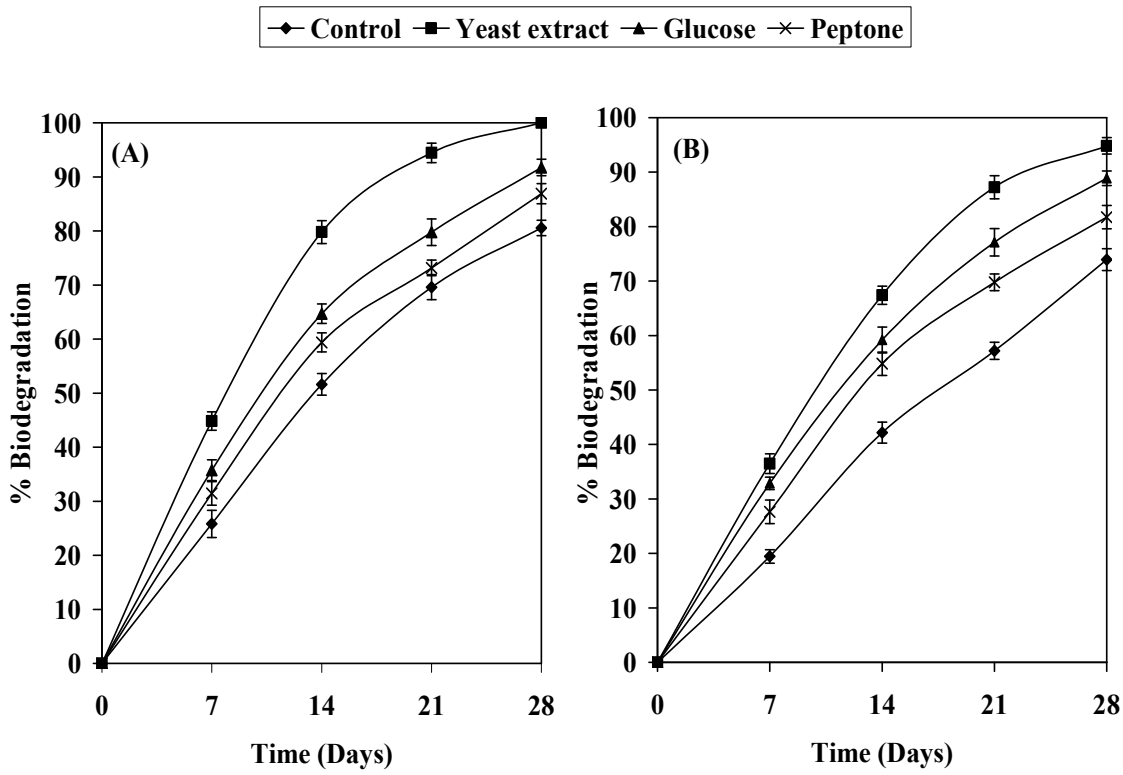

Fig. 5: Effect of different carbon sources on biodegradation of phenanthrene by (A) *P. chrysosporium* and (B) *P. citrinum* (50 ppm).

This publication is licensed under a Creative Commons Attribution 4.0 International License

were supplemented. The results of the present study are similar to organic carbon source supplementation and the presence of co-substrates, which improve the transformation of PAHs, as observed by Hesnawi & Adbeib (2013). In the present study, almost 19 to 20 percent degradation rate was higher with yeast extract than the degradation without the addition of a carbon source. The addition of a carbon source is required for the initial growth of microorganisms as the energy required for microbial growth is not provided by PAHs alone as a carbon source (Cerniglia & Sutherland 2010).

Biodegradation of PAHs Mixture

Although many organisms degrade individual PAHs, to be effective in PAH bioremediation, they must metabolize them in the environment as a complex mixture (Haritash & Kaushik 2009). Therefore, it is important to study the effect of PAH degradation in a synthetic liquid medium and artificially spiked soil at optimized conditions with the addition of 5 $g.L^{-1}$ yeast extract as a carbon source.

The experiments presented here were to measure fungal degradation of a mixture of five PAHs for 28 days incubation period (sampled at days 0, 7, 14, 21, and 28). There were no significant reductions in PAHs with uninoculated and autoclaved killed controls. Only 1 to 2 % total degradation of PAHs by both the control with both fungal strains. All results presented in this study were not corrected with their controls.

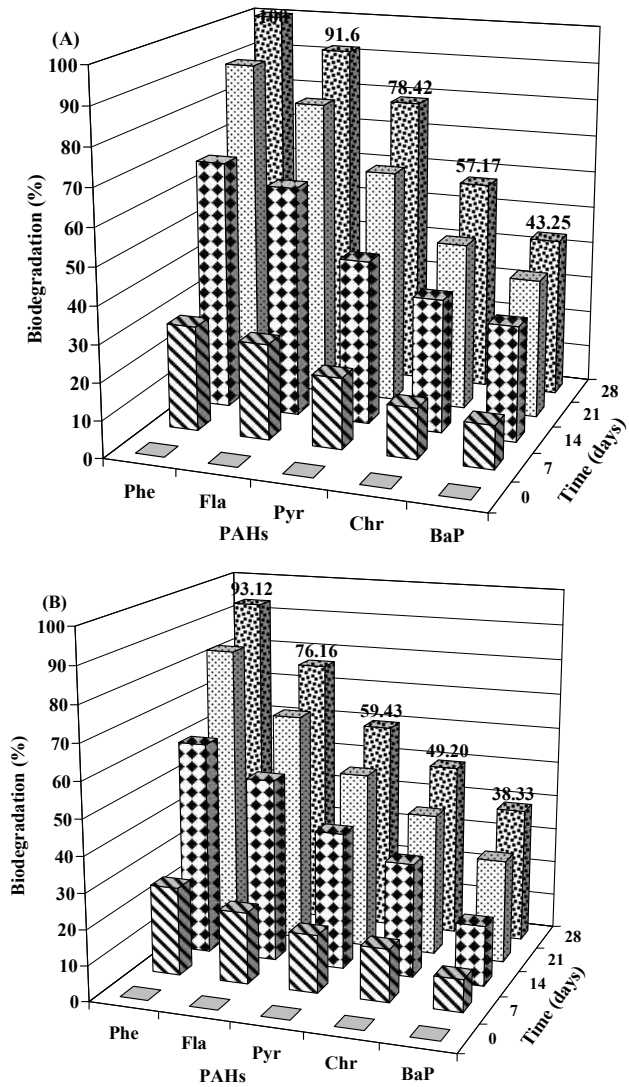

Fig. 6: The percentage biodegradation of individual PAHs in a synthetic mixture in liquid media after 28 days incubation period with (A) *P. chrysosporium* and (B) *Penicillium citrinum* in liquid media. Phen, Phenanthrene; Flu, Fluoranthene; Pyr, Pyrene; Chr, Chrysene; BaP, Benzo(a)pyrene.

This publication is licensed under a Creative Commons Attribution 4.0 International License

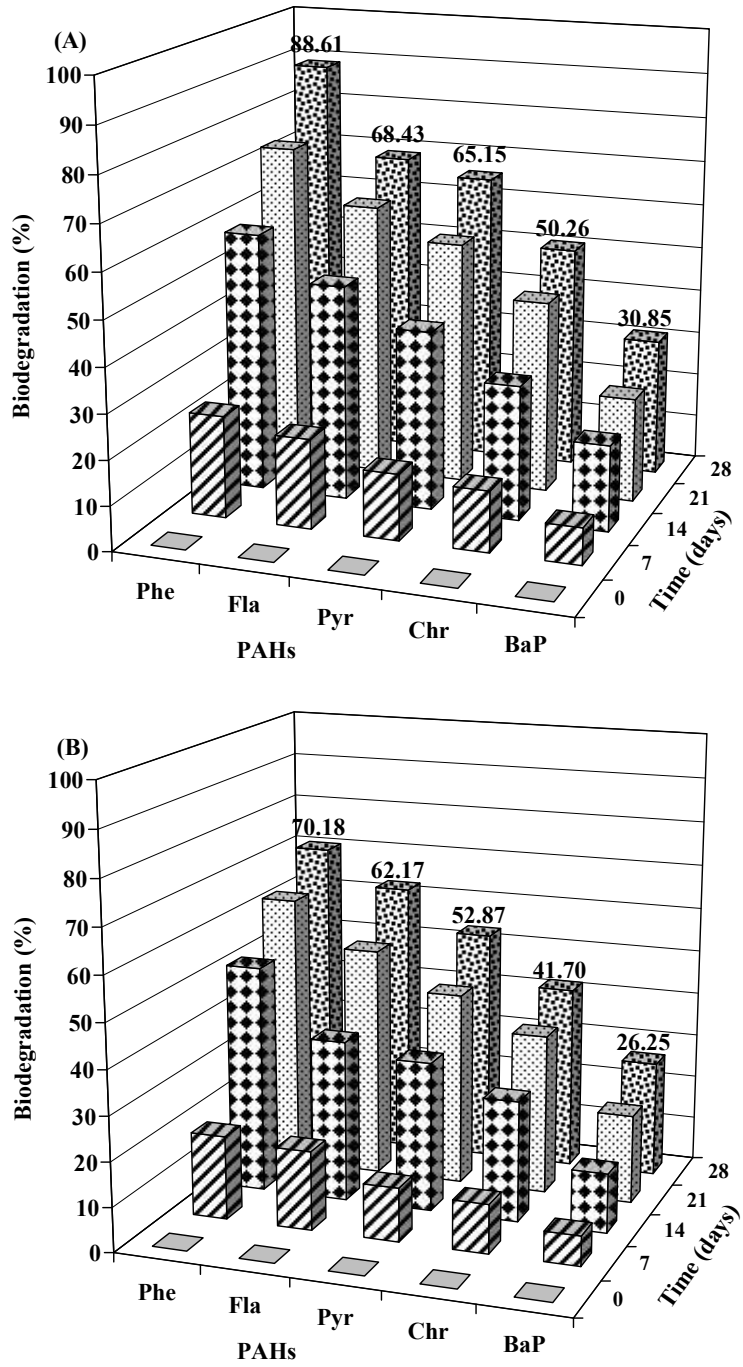

Fig. 7: The percentage biodegradation of individual PAHs in a synthetic mixture in spiked soil after 28 days incubation period with (A) *P. chrysosporium* and (B) *Penicillium citrinum* in spiked soil.

In liquid medium: In liquid medium, the degradation of PAHs mixture with *P. chrysosporium* was 100%, 91.60%, 78.42%, 57.17%, and 43.25% of phenanthrene, fluoranthene, pyrene, chrysene, and benzo(a)pyrene, respectively, as shown by Fig. 6 A after 28 days, indicating the highly efficient strain. Fig. 6 B showed 93.12%, 76.16%, 59.43%, 49.20%, and 38.33% of phenanthrene, fluoranthene, pyrene, chrysene, and benzo(a)pyrene, respectively, degraded by *P. citrinum* within 28 days. Both fungal strains show less efficiency towards degradation of four to five rings PAHs

This publication is licensed under a Creative Commons Attribution 4.0 International License

(chrysene and benzo(a)pyrene). PAHs of higher molecular weight have lower aqueous solubility, which in turn reduces the bioavailability (Ghosal et al. 2016), thus lowering the biodegradation potential. Pyrene was more rapidly degraded than benzo(a)pyrene with *Trichoderma sp*., *Aspergillus niger*, and *Fusarium sp*. (Wang et al. 2008). Quintero et al. (2008) observed 42% degradation of γ-HCH in a liquid medium while 8 to 17% degradation in sandy soil using *B*. *adusta*. The biodegradation of higher molecular weight PAHs is slower than low molecular weight PAHs might be due to their low water solubility, lower uptake into the cell, not induction of degradative enzymes, and insufficient energy yield required for microbial growth (Cerniglia & Sutherland 2010).

In spiked soil: The experiment was carried out in a liquid medium, and optimal conditions were used to ascertain the potential of biodegradation and the influence of different factors such as pH and temperature. However, it is difficult to extrapolate the results of such studies to the field because of strong ecological competition from soil microflora and variable environmental conditions (Diplock et al. 2009, Kadri et al. 2017). To better simulate field conditions, while retaining experimental control, microcosm can be used. These generally comprise small bioreactors, usually jars or Erlenmeyer flasks, where fungal strains grown on a mixture of soil are artificially or naturally contaminated with additional nutrient sources (Canet et al. 1999).

In this study, firstly, the degradation rate of PAHs in liquid media was carried out, followed by spiked soil samples having a mixture of phenanthrene, fluoranthene, pyrene, chrysene, and benzo(a)pyrene. Fig. 7 A, B showed that *P. chrysosporium* was more efficient than *P. citrinum* in removal of five PAHs: 88.61 and 70.18% phenanthrene, 68.43 and 62.17% fluoranthene, 61.15% and 52.87% pyrene, 50.26% and 41.70% chrysene and 30.85 and 26.25% benzo(a)pyrene, respectively (initial concentration = 100% of individual PAH in the soil for experiment). Within 14 days, the removal of phenanthrene was 67.15 and 60.31% with *P. chrysosporium* and *P. citrinum*, respectively, after the degradation rate was slowed down. Phenanthrene degradation was more with both isolates in both cases because in the adaptation experiment, individual microbial culture was enriched with phenanthrene for three months and then with a combination of the five PAHs for another next three months. Similarly, Yuan et al. (2002) reported faster degradation of phenanthrene as compared to other PAHs due to the enrichment of consortium in phenanthrene for three years and in combination with other PAHs for one year. The degradation of chrysene and benzo(a) pyrene was less might be due to their low water solubility.

The degradation pattern was very interesting as in the first two weeks removal rate of PAHs was high, followed by lower removal rates in the coming two weeks in all experiments of this work. The percent degradation of PAHs increased with increasing incubation time, but the rate of degradation decreased. This might be due to nutrient limitations as one possibility for a decreased rate of removal. Kalantary et al. (2014) reported the bacterial degradation of phenanthrene in soil was enhanced by the addition of a suitable combination of mineral nutrients in the system. In the present study, more PAH is eliminated in a liquid medium than in soil. This might be due to different mass transfers between soil and liquid. As a result of this, PAH showed a much lower rate of metabolism in soil than in liquid media (Wang et al. 2009). Bioavailability is one of the critical factors involved in the bioremediation of hydrophobic pollutants as they tend to sorb on mineral surfaces and organic matter (Ghosal et al. 2016). This results in a reduction in bioavailability of pollutants, thus limiting their bioremediation in soil. The present study indicated that mineralization of phenanthrene was higher in liquid media as compared to soil and biodegradability might be reduced due to sorption of PAHs.

CONCLUSION

This study was conducted to investigate the ability of isolated fungal strains for PAH removal from liquid and spiked soil. Favorable conditions for the development of microorganisms and the addition of carbon sources play a significant role in PAH degradation. Results showed that variations influence phenanthrene biodegradation in pH, temperature, substrate concentrations, and the supplementation of carbon sources. The optimal conditions for PAH biodegradation were determined as pH 7.0 and 30^{o}C. The addition of all three carbon sources enhanced the degradation rate, but yeast extract showed promising results. *P. chrysosporium* showed higher degradation potential than *P. citrinum*. The maximum degradation rate was obtained in liquid medium than spiked soil.

ACKNOWLEDGEMENTS

The authors are delighted to express their gratitude and sincerest thanks to the Dean of the Central Instrumentation Laboratory (CIL) at Guru Jambheshwer University of Science and Technology, Hisar, for the cartography unit.

REFERENCES

Abdel-Shafy, H.I. and Mansour, M.S.M. 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt. J. Petro., 25: 107-123.https://doi.org/10.1016/j.ejpe.2015.03.011

Al-Hawash, A.B., Alkooranee, J.T., Zhang, X. and Ma, F. 2018. Fungal degradation of polycyclic aromatic hydrocarbons. Int. J. Pure App. Biosci., 6(2): 8-24. doi: http://dx.doi.org/10.18782/2320-7051.6302

This publication is licensed under a Creative Commons Attribution 4.0 International License

Alekseev, I. and Abakumov, E. 2021. Polycyclic aromatic hydrocarbons, mercury, and arsenic content in soils of Larsemann Hills, Pravda Coast, and Fulmar Island, Eastern Antarctica. Bull. Environ. Contam. Toxicol., 106(2): 278-288. doi: 10.1007/s00128-020-03063-w.

Babu, A.G., Reja, S.I., Akhtar, N., Sultana, M., Deore, P.S. and Ali, F.I. 2019. Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs): Current Practices and Outlook. In Arora P. (ed), Microbial Metabolism of Xenobiotic Compounds. Microorganisms for Sustainability, Springer, Singapore, pp. 46-80. Online ISBN 978-981-13-7462-3. 10.1007/978-981-13-7462-3_9

Bishnoi, K., Sain, U., Kumar, R., Singh, R. and Bishnoi, N.R. 2009. Distribution and biodegradation of polycyclic aromatic hydrocarbons in contaminated sites of Hisar (India). Indian J. Exp. Biol., 47: 210-217.

Bishnoi, K., Kumar, R. and Bishnoi, N.R. 2008. Biodegradation of polycyclic aromatic hydrocarbons by white rot fungi *Phanerochaete chrysosporium* in sterile and unsterile soil. J. Sci. Indust. Research., 67: 538-542.

Bishnoi, N.R., Mehta, U. and Pandit, G.G. 2006. Quantification of polycyclic aromatic hydrocarbons in fruits and vegetables using high-performance liquid chromatography. Indian J. Chem. Technol., 13: 30-35.

Bright, A., Li, F., Movahed, M., Shi, H. and Xue, B., 2023. Chronic exposure to lowmolecular-weight polycyclic aromatic hydrocarbons promotes lipid accumulation and metabolic inflammation. Biomolecules, 13:196. https://doi.org/10.3390/biom13020196

Canet, R., Lopez-Real, J.M. and Beck, A.J. 1999. Overview of polycyclic aromatic hydrocarbon biodegradation by white-rot fungi. Land Contam. Reclam., 7: 191-197.

Cerniglia, C.E. and Sutherland, J.B., 2010. Degradation of Polycyclic Aromatic Hydrocarbons by Fungi. In Temmis, K.N. (ed), Handbook of Hydrocarbon and Lipid Microbiology, Springer Berlin, Heidelberg, pp 2079-2110.

Diplock, E.E., Mardlin, D.P, Killham, K.S. and Paton, G.I. 2009. Predicting bioremediation of hydrocarbons: Laboratory to field scale. Environ. Pollu., 157: 1831-1840. https://doi.org/10.1016/j.envpol.2009.01.022

Etim, E.U., Ola, M.I. and Owoade, R.O. 2022. Bioremediation of total polycyclic aromatic hydrocarbon contaminated soil using nitrified sawdust and *Pseudomonas aeruginosa*. J. Environ. Protect., 13: 427-43. 10.4236/jep.2022.136027

Ghosal, D., Ghosh, S., Dutta, T.K. and Ahn, Y. 2016. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Front. Microbiol., 7: 1369. https://doi.org/10.3389/fmicb.2016.01369

Gu, H., Yan, K., You, Q., Chen, Y., Pan, Y., Wang, H., Wu, L. and Xu, J. 2021. Soil indigenous microorganisms weaken the synergy of Massilia sp. WF1 and Phanerochaete chrysosporium in phenanthrene biodegradation. Sci. Total Environ., 781: 146655. https://doi.org/10.1016/j.scitotenv.2021.146655

Gupta, H. and Kumar, R. 2020. Distribution of selected polycyclic aromatic hydrocarbons in urban soils of Delhi, India. Environ. Technol. Innovat., 17(7): 100500. 10.1016/j.eti.2019.100500

Haritash, A.K. and Kaushik, C.P. 2009. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. J. Hazard. Mater., 169: 1-15. https://doi.org/10.1016/j.jhazmat.2009.03.137

Hesnawi, R. and Adbeib, M. 2013. Effect of nutrient source on indigenous biodegradation of diesel fuel contaminated soil. APCBEE Proced., 5: 557-561. https://doi.org/10.1016/j.apcbee.2013.05.093

Ibrahim, M., Makky, E.A., Azmi, N.S. and Ismail, J. 2018. Optimization parameters for Mycobacteria confluentis biodegradation of PAHs. MATEC Web Conf., 150: 06035. https://doi.org/10.1051/matecconf/201815006035

Jacques, R.J.S., Okeke, B.C., Bento, F.M., Teixeira, A.S., Peralba, M.C.R. and Camargo, F.A.O. 2008. Microbial consortium bioaugmentation of polycyclic aromatic hydrocarbons contaminated soil. Biores. Technol., 9(7): 2637-43. doi: 10.1016/j.biortech.2007.04.047.

Kadri, T., Rouissi, T., Brar, S.K., Cledon, M., Sarma, S. and Verma, M. 2017. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. J. Envirn. Sci., 51: 52-74. https://doi.org/10.1016/j.jes.2016.08.023

Kalantary, R.R., Mohseni-Bandpi, A., Esrafili, A., Nasseri, S., Ashmagh, F.R., Jorfi, S. and Ja'fari, M. 2014. Effectiveness of biostimulation through nutrient content on the bioremediation of phenanthrene contaminated soil. J. Environ. Health Sci. Engg., 12(1): 24. DOI:10.1186/2052-336X-12-24

Liu, S.H., Zeng, G.M., Niu, Q.Y., Liu, Y., Zhou, L., Jiang, L.H., Tan, X.F., Xu, P., Zhang, C. and M. Cheng 2017. Bioremediation mechanisms of combined pollution of PAHs and heavy metals by bacteria and fungi: A mini-review. Biores. Technol., 224: 25-33. https://doi.org/10.1016/j.biortech.2016.11.095

Malik, A., Verma, P., Singh, A.K. and Singh, K.P. 2011. Distribution of polycyclic aromatic hydrocarbons in water and bed sediments of the Gomti River, India. Environ. Monit. Assess., 172(1-4): 529-545. doi: 10.1007/s10661-010-1352-4.

Masih, A., Saini, R., Singhvi, R. and Taneja, A. 2010. Concentrations, sources, and exposure profiles of polycyclic aromatic hydrocarbons (PAHs) in particulate matter (PM_{10}) in the north-central part of India. Environ. Monit. Assess., 163: 421-431.

Mineki, S., Suzuki, K., Iwata, K., Nakajima, D. and Goto, S. 2015. Degradation of polyaromatic hydrocarbons by fungi isolated from soil in Japan. Polycycl. Aromat. Compd., 128-120 :(1)35. https://doi.org/10406638.2014.937007/10.1080

Okere, U.V. and Semple, K.T. 2012. Biodegradation of PAHs in pristine soils from different climatic regions. Bioremed. Biodegrad., 3: 1-11. doi: 10.4172/2155-6199.S1-006.

Paris, A., Ledauphin, J., Poinot, P. and Gaillard, J.L. 2018. Polycyclic aromatic hydrocarbons in fruits and vegetables: Origin, analysis, and occurrence. Environ. Pollut., 234: 96-106. https://doi.org/10.1016/j.envpol.2017.11.028

Qi, Y., Wang, C., Lv, C., Lun, Z. and Zheng, C. 2017. Removal capacities of polycyclic aromatic hydrocarbons (PAHs) by newly isolated strain from oilfield-produced water. Int. J. Eviron. Res. Pub. Health, 14(2): 215. doi: 10.3390/ijerph14020215.

Quintero, J.C., Moreira, M.T., Feijoo, G. and Lema, J.M. 2008. Screening of white rot fungal species for their capacity to degrade lindane and other isomers of hexachlorocyclohexane (HCH). Cien. Inv. Agr., 35(2): 159-167. http://dx.doi.org/10.4067/S0718-16202008000200005

Saraswathy, A. and Hallberg, R. 2002. Degradation of pyrene by indigenous fungi from a former gasworks site. FEMS Microbiol. Lett., 210(2): 227-232. 10.1111/j.1574-6968.2002.tb11185.x.

Sawulski, P., Boots, B., Clipson, N. and Doyle, E. 2015. Differential degradation of polycyclic aromatic hydrocarbon mixtures by indigenous microbial assemblage in soil. Lett. Appl. Microbiol., 61(2):199-207. doi: 10.1111/lam.12446.

Shankhwar, A.K. and Paliwal, R.2021. Fungi in bioremediation of soil: Mycoremediation of PAHs compounds. In Sharma, V.K., Shah, M.P., Parmar, S. and Kumar, A. (eds), Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-technology. Academic Press, Cambridge, MA, pp. 157-170. doi:10.1016/B978-0-12-821925-6.00008-3.

Singh, P. and Tiwary, B.N. 2017. Optimization of conditions for polycyclic aromatic hydrocarbons (PAHs) degradation by *Pseudomonas stutzeri* P2 isolated from Chirimiri coal mines. Biocata. Agri. Biotechnol., 10: 20-29. https://doi.org/10.1016/j.bcab.2017.02.001

Sonwani, R.K., Giri, B.S., Singh, R.S. and Rai, B.N. 2019. Studies on optimization of naphthalene biodegradation using surface response methodology: kinetic study and performance evaluation of a pilot scale integrated aerobic treatment plant. Process Saf. Environ. Prot., 132: 240-248. doi:10.1016/j.psep.2019.10.004

This publication is licensed under a Creative Commons Attribution 4.0 International License

US EPA 2002. Polycyclic Organic Matter. Retrieved January 20, 2021, from http://www.epa.gov/ttn/atw/hlthef/polycycl.html.

Thacharodi, A., Hassan, S., Singh, T., Mandal, R., Chinnadurai, J., Khan, H.A., Hussain, M.A., Brindhadevi, K. and Pugazhendhi, A., 2023. Bioremediation of polycyclic aromatic hydrocarbons: An updated microbiological review. Chemosphere, 328: 138498. https://doi.org/10.1016/j.chemosphere.2023.138498

Ting, W.T.E., Yuan, S.Y., Wu, S.D. and Chang, B.V. 2011. Biodegradation of phenanthrene and pyrene by *Ganoderma lucidum*. Int. Biodeterior. Biodegrad., 65: 238-242. doi: 10.1016/j.ibiod.2010.11.007.

Wang, H., Lou, J. and Xu, J. 2016. Efficient biodegradation of phenanthrene by a novel strain *Massilia sp*. WF1 isolated from PAH-contaminated soil. Environ. Sci. Pollut. Res., 23(13):13378-88. doi: 10.1007/s11356-016-6515-6.

Wang, C., Sun, H., Li, J., Li, Y. and Zhang, Q. 2009. Enzyme activities during degradation of polycyclic aromatic hydrocarbons by white rot fungus *Phanerochaete chrysosporium* in soils. Chemos., 77(6): 733-738. https://doi.org/10.1016/j.

Wang, X. X., Gong, Z., Li, P., Zhang, L. and Hu, X. 2008. Degradation of pyrene and benzo(a)pyrene in contaminated soil by immobilized fungi. Environ. Engg. Sci., 25: 677-684. https://doi.org/10.1089/ees.2007.0075.

Yuan, S.Y., Shiung, L.C. and Chang, B.V. 2002. Biodegradation of polycyclic aromatic hydrocarbons by inoculated microorganisms in soil. Bull. Environ. Contam. Toxicol., 69(1):66-73. doi: 10.1007/s00128-002-0011-z.

Zhong Y., Luan T., Lin L., Liu H. and Tam N. 2011. Production of metabolites in the biodegradation of phenanthrene, fluoranthene, and pyrene by the mixed culture of *Mycobacterium* sp. and *Sphingomonas* sp. Biores. Tech., 102(3):2965-72. doi: 10.1016/j.biortech.2010.09.113.

This publication is licensed under a Creative Commons Attribution 4.0 International License