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Comprehensive Kinetic Study of Phenanthrene and Naphthalene Biodegradation by Soil Fungi from Urban Areas Using Mycoremediation Approaches

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Abstract

Soil contamination by polycyclic aromatic hydrocarbons (PAHs) poses serious environmental and health risks. PAHs are ubiquitous pollutants composed of multiple aromatic rings and are hazardous to the environment due their mutagenic and carcinogenic effects. Among PAHs, naphthalene and phenanthrene are focused on this research due to their prevalence in contaminated environments. Bioremediation denotes the application of microorganisms to eradicate or diminish the level of hazardous waste at contaminated sites. In this research, mycoremediation technique is employed where fungal species are utilized to degrade PAHs. This study aimed to investigate the phenanthrene (PHE) and naphthalene (NAP) biodegradation potential of different fungal strains isolated from soil samples of three distinct urban areas namely Galle (6°02'.78"N 80°12'35.26"E), Jaffna (9°40'0"N 80°02'0"E) and Colombo (6°28'.0"N 79°58'0"E). Morphologically different fungal strains were isolated using serial dilution technique. Isolated ones were screened for their capacity to breakdown PHE and NAP in bushnell hass agar supplemented with both PAHs at 30°C for 3 days. Molecular identification was conducted, and accession numbers were obtained from NCBI. The best degraders underwent kinetic analysis. Cytotoxic experiments were carried out using Brine shrimp lethality assay to assess the toxicity, and a phylogenetic tree was created to determine evolutionary relationships. Eight morphologically different fungal strains were isolated followed by a series of screening processes to screen for their ability to degrade PAHs, NAP and PHE. Kinetic Analysis demonstrated only two different fungal strains G1-1 (Galle) and J1-2 (Jaffna) as the best PAH degraders among the eight strains. These fungal strains were identified as *Trichoderma harzianum* isolate (PV225528) and *Talaromyces verruculosus* (PV225531) based on ITS sequence data. *Trichoderma harzianum* (PV225528) showed the highest degradation rate for NAP and *Talaromyces verruculosus* (PV225531) showed the highest degradation rate for PHE. Toxicity Assay using *Artemia salina* indicated reduced toxicity compared to 100 ppm PAHs supplemented seawater after the degradation of PAHs by *Trichoderma harzianum* PV225528 and *Talaromyces verruculosus* PV225531. The study highlights the significance of fungal bioremediation as a sustainable and effective approach for degrading PAHs in contaminated soils.

Keywords: Phenanthrene, Naphthalene, PAHs, Biodegradation, *Trichoderma harzianum, Talaromyces verruculosus*

1. Introduction

1.1 Soil pollution

Soil pollution refers to contamination of soil by higher than normal concentration of pollutants such as heavy metals, PAHs, microplastics, and toxic organic chemicals such as pesticides. It is a growing environmental concern that has adverse effects on ecosystems and human health[1]. Human activities such as urbanization, improper waste disposal, industrialization and use of agrochemicals are primary sources of soil pollution[2].

1.2 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are organic compounds that are composed of two or more fused aromatic rings of carbon and hydrogen atoms. They are present in crude oil and are primarily produced by the partial combustion of fossil fuels and coals. In general, PAHs are solids that are colorless, white or pale yellow-green and they have a mild pleasant odor including examples such as anthracene, naphthalene and phenanthrene. They are ubiquitous in the environment and pose potential mutagenic, carcinogenic and toxic effects on both humans and other living organisms. Based on the numbers of rings present in the compound, they are classified into light light-molecular weight (LMW) and high molecular weight (HMW) PAHs. LMW have two or three aromatic rings while HMW consists of four or more aromatic rings[3]. PAHs are commonly found in urban areas due to vehicular emissions and poor dispersion of pollutants[4]. The US Environmental Protection Agency (US EPA) has listed 16 PAHs as priority pollutants due to their dangerous properties. Humans are exposed to PAHs via inhalation, ingestion or even dermal contact. Several adverse health effects such as diabetes, oxidative stress, infertility, oxidative stress and poor fetal development are associated with exposure to PAHs[5].

1.3 Naphthalene (NAP)

NAP is classified as a HWM PAH and is commonly found in coal, mothballs, disinfectant and during polyvinyl chloride production. Various sources of naphthalene emissions include industrial processes, combustion and the distillation and crystallization of coal tar fractions. It has a molecular formula of $C_{10}H_8$ and is the most volatile PAH. It is a ubiquitous pollutant[6]. Naphthalene can effectively reduce the individual density and population of soil fauna. High levels of NAP can destroy RBCs in humans resulting in hemolytic anemia. Studies in animals have shown that inhaling air contaminated with naphthalene results in nose and lung tumors. Due to this, the US Department of Health and human services and the International Agency for Research on Cancer have concluded that NAP causes cancer[7].

1.4 Phenanthrene (PHE)

PHE is classified as a LWM PAH and has a molecular formula of $C_{14}H_{10}$. The incomplete combustion of various organic materials such as wood and fossil fuels releases PHE. Once PHE is released, it adheres itself to airborne particles and settles on the surface of soil and water. Urban populations are exposed to PHE by ingesting contaminated food and water, inhaling contaminated air and dermal contact. It is used in making dyes, production of pharmaceutical products, plastics and explosives. A forward mutation was found in human lymphoblast cells that were metabolically activated and also exposed to 9 ug/mL of PHE[8]. PHE pollution decreases the microbial community's diversity and increased PHE levels limits microbial metabolism resulting in decline in soil fertility. Prolonged exposure to PHE is associated to adverse health effects including carcinogenic effects[9].

1.5 PAHs distribution in urban areas

Urban areas serve as hotspots for PAH contaminants due to increased anthropogenic activities such as industrial activities, vehicular emissions, and improper waste disposal. PAHs contaminants enter into soils through atmospheric deposition and direct spillage of petroleum. With rapid urbanization, soil contamination by PAHs has become a growing concern, highlighting the need for effective remediation techniques. Their degradation is essential to reduce soil pollution and in preventing long term ecological damage[10].

1.6 Bioremediation

Bioremediation uses microorganisms to reduce hazardous waste concentrations in contaminated sites and it is carried out in non-sterile environments with various microorganisms, with fungi playing a central role in degradation[11]. Degradation of the pollutants is facilitated by the microorganism's enzymatic metabolic pathways. Factors such as type of the soil, pH, concentration of contaminants and the ability of microorganism to bind to the pollutants influences the effectiveness of bioremediation[12]. It is a sustainable approach to control environmental pollution and manage waste. Mycoremediation

is a form of bioremediation in which enzymes produced by fungi instead of bacteria are used to break down pollutants and restore balance to the ecosystem. There is a wide variety of organisms that are capable of degrading LMW PAHs such as NAP and PHE and acenaphthene. Various fungal species such as *Penicillium, Aspergillus, Trichoderma* and *Rhizopus* are effective in degrading a wide range of pollutants including PAHs. Fungi produce lignolytic enzymes such as laccase, lignin peroxidase and manganese peroxidase which enables fungi to degrade the PAHs. These enzymes work in a broad and non-specific way making them promising strategies in the removal of PAHs. In addition to extracellular lignolytic enzymes, Cytochrome P-450 Monooxygenase has been shown to aid in PAH breakdown. Certain lignolytic fungi have the ability to break down both LMW as well as HMW PAHs. Mycoremediation is an eco-friendly, cost-effective and sustainable approach for preventing the rising issue of soil pollution[13]. This study aimed to investigate the phenanthrene (PHE) and naphthalene (NAP) biodegradation kinetics of different fungal strains isolated from soil samples of three distinct urban areas in Sri Lanka.

Methodology

2.1 Sample collection

A 50g of soil samples were aseptically collected from distinct locations across the country, i.e., Jaffna-J (9°40'0"N 80°02'0"E), Colombo-C (6°28'0"N 79°58'0"E) and Galle-G (6°02'.78"N 80°12'35.26"E). Samples were collected from a depth of 5-10 centimeters in urban areas using sterile rules and transferred into sterile zip-lock bags. Samples were collected and transferred under ambient conditions and stored at 4°C in the refrigerator.

2.2 Spread plate technique

A total 5g of each soil sample was weighed using an analytical balance and transferred into individually labelled conical flasks. Each soil sample was washed separately with 50 ml of 0.9% NaCl solution by mixing gently ensuring an even distribution of soil particles. The supernatant was followed by a tenfold serial dilution was performed. Potato Dextrose Agar (PDA) medium was prepared, autoclaved and poured into sterile petri dishes that had been previously sterilized by keeping it under the UV light. After the medium solidified, 100 µL of the diluted soil suspension from the selected dilution factors 10^{-1}, 10^{-4} and 10^{-8} were pipetted onto the surface of PDA plates. Then the inoculum was evenly spread across the plate using a sterile glass spreader. The petri plates were then incubated at 30°C for 3 days.

2.3 Isolation of fungal strains and starvation

Fungal colonies with distinct morphologies were selected from different PDA plates and sub-cultured onto fresh PDA plates to obtain pure cultures. The petri plates were then incubated at 30°C temperature for 5 days. Bushnell Haas Broth (BHB) along with Bacteriological Agar (BA) was prepared, autoclaved and poured into sterile petri plates that were partitioned into four divisions and allowed to set. The purified fungal strains were starved by inoculating them into the center of each partition in the Bushnell Hass Agar (BHA) medium. The plates were incubated under 30°C for 3 days to determine their survival and adaptability.

2.4 PAHs spiking

BHA media was prepared, autoclaved and poured into sterile petri plates that were partitioned into four divisions and allowed to set. The petri plates were divided, with eight designated to NAP and eight for PHE. A 100-ppm solution of NAP and PHE was prepared separately. On their respective plates, using a cotton swab, PHE and NAP solution were swabbed. Four divisions were created in each petri plate, with two sections inoculated with the same sample in the center while the other two sections were inoculated with a different sample. The plates were then incubated for three days at 30°C.

2.5 DNA Extraction

Fungal cultures were scraped and crushed using mortar and pestle separately. Then the samples were transferred into 1.5 mL microcentrifuge tubes which were vortexed for 1-3 seconds and incubated at 65°C for 15 minutes. Then 3 µl of RNase Solution was added to each microcentrifuge tube containing the cell lysate and was mixed by inverting the tube containing the samples 2–5 times. And incubated at 37°C for 15 minutes. The samples were then allowed to cool to room temperature for 5 minutes

before proceeding. A total of 200 µl of Protein Precipitation Solution was added and vortexed vigorously at high speed for 20 seconds and then centrifuged for 3 minutes at 14,000 rpm. The precipitated proteins formed a tight pellet. To another set of 1.5 ml microcentrifuge tubes 600 µl of room temperature isopropanol were pipetted. The supernatant containing the DNA was carefully removed, transferred to the tubes containing the isopropanol and was mixed by inversion until thread-like strands of DNA were formed. The samples were centrifuged at 14,000 rpm for 1 minute at room temperature. The supernatant was decanted carefully. Then 600µl of 70% ethanol was added and was inverted several times to wash the DNA. The samples were centrifuged at 13,000–16,000 rpm for 1 minute at room temperature. The ethanol was drawn out using a micropipette. A total of 100µl of DNA Rehydration Solution was added to the samples and were incubated at 65°C for 1 hour. Periodically the solution was mixed by gently tapping the tube. The solution was incubated overnight at 4°C. The DNA was stored at 2–8°C.

2.6 PCR

After extracting DNA from the selected fungal strains that could degrade the PAHs, PCR was performed. For each sample, 8.5 µL of Nuclease-free water, 12.5 µL of Go Taq® green master mix, 1 µL from each fungal primers ITS1 forward primer (5'TCC GTA GGT GAA CCT GCC G3') and ITS4 reverse primer (5'TCC TCC GCT TAT TGA TAT GC 3') was added to each PCR tube containing 2 µL of the extracted DNA samples. The PCR samples were loaded into the PCR machine and the following conditions were set: initial denaturation for 5 mins at 94 °C, denaturation at 94° C for 30 seconds, annealing for 1 min at 52 °C, extension for 1 min at 72 °C for 35 cycles and final extension for 5 mins at 72 °C. Once the conditions were set, the machine was allowed to run.

2.7 Gel electrophoresis

A 1.5% agarose gel was prepared, heated and then transferred to a biosafety cabinet to cool down. Once cooled, 4 µL of ethidium bromide (EtBr) was added to the solution. The gel solution was poured into a cassette and allowed to set. It was then placed in the chamber and a 1X TAE running buffer was added which was prepared. Using a micropipette, 10 µL of 1KB DNA ladder was loaded into the first well followed by 10 µL of PCR samples into the other wells. The electrophoresis was run at 65 volts for 40 mins. The gel was then visualized under UV light to visualize the bands.

2.8 DNA sequencing

Sequencing of the DNA was performed via the sanger sequencing method and the sequences were submitted to the NCBI BLAST tool to determine the identity of each isolate. The resultant accession numbers were obtained from NCBI GenBank. Then the phylogenetic tree was constructed using mega software.

2.9 Kinetic Analysis

Kinetic analysis was performed. For eight days, 16 test tubes were prepared for each sample, eight tubes designated for NAP while the other eight were designated for PHE. This procedure was repeated for all other samples. BHB media was prepared and autoclaved. Once autoclaved, the media was divided into two different portions, one portion was supplemented with 100 ppm of NAP solution while the other portion was supplemented with 100 ppm of PHE solution. A total of 10 mL from both the media portions were transferred to their corresponding test tubes. A total of 500 µL of methylene blue solution was added to each test tube followed by inoculation of samples. After 24 hours, the first test tube was taken from each sample set (NAP and PHE) and centrifuged. The supernatant was measured using a spectrophotometer to determine the absorbance value. This procedure was repeated every 24 hours for each sample for eight days.

2.10 Statistical and Phylogenetic Analysis

Anova single factor test was conducted to determine whether there are significant differences in the degradation rate of PHE among the three fungal strains, i.e., C1-3, G1-1 andJ1-2.

The phylogenetic tree of the selected strains was designed using MEGA11 software version 11.0.13.

2.11 Toxicity Assay

Brine shrimps *(Artemia salina)* cysts were allowed to hatch for 48 hours in 500 mL filtered seawater. Two test tubes were taken for each sample, one for PHE and the other for NAP. BHB was prepared and autoclaved. Once autoclaved, the media was divided into two different portions, one portion was supplemented with 100 ppm of NAP solution while the other portion was supplemented with 100 ppm of PHE solution. A total of 10 mL from both the media portions were transferred to their corresponding test tubes. The test tubes were inoculated and incubated at room temperature for eight days. On the 8th day the samples were centrifuged and transferred into petri plates. Ten nauplii were transferred to each Petri plate using a Pasteur pipette. The mortality readings were recorded hourly for four hours, followed by observation at 24 hours and 48 hours.

2.11 Microscopic analysis

Fungal colonies were selected and subjected to morphological characterization using the Scotch tape method, as previously described[14]. A small piece of transparent adhesive tape (Sellotape) was gently pressed onto the surface of the fungal colony to collect fungal structures, including hyphae and conidia.

The tape was then mounted onto a clean glass microscope slide containing a drop of lactophenol cotton blue, a staining and mounting medium that enhances visualization by staining chitinous components of the fungal cell wall. The slide was examined under a light microscope at 40× magnification to observe key morphological features such as hyphal organization, spore type, arrangement, and reproductive structures. These characteristics were used to aid in the preliminary identification and differentiation of fungal taxa.

2. Results and Discussion

3.1 Sample collection and Isolations

Soil samples were collected from three distinct regions as pollution is abundant in such areas and also to cover geographical diversity Soil sample collection from distinct regions also provides a diverse microbial profile as different fungal species have varying sensitivity to pollutants. The ten-fold dilution series reduced the overgrowth of colonies thereby allowing for the isolation of individual distinct colonies (figure 1).

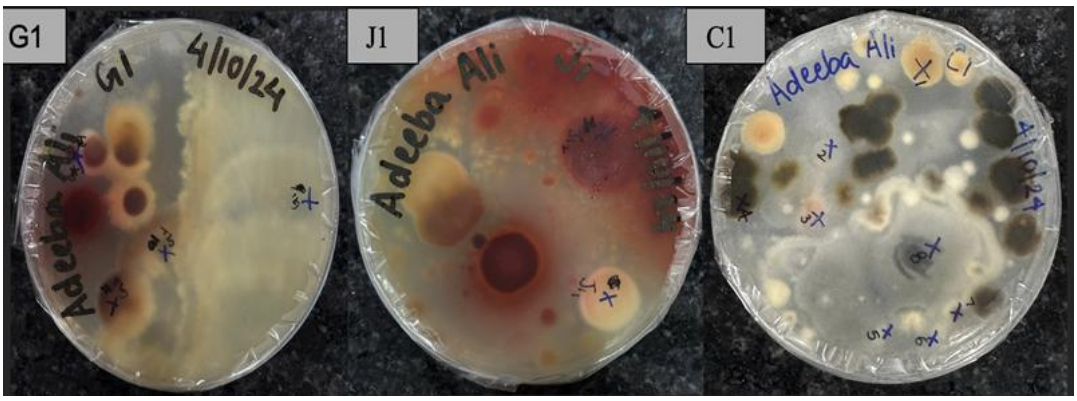

Figure 1: Morphologically different Fungal colonies from Galle (G1), Jaffna (J1) and Colombo (C1).

3.2 Subculturing

Pure fungal cultures were observed after sub culturing. PDA is effective for fungal isolations as it provides an optimal nutrient-rich environment for fungal growth (Figure 2).

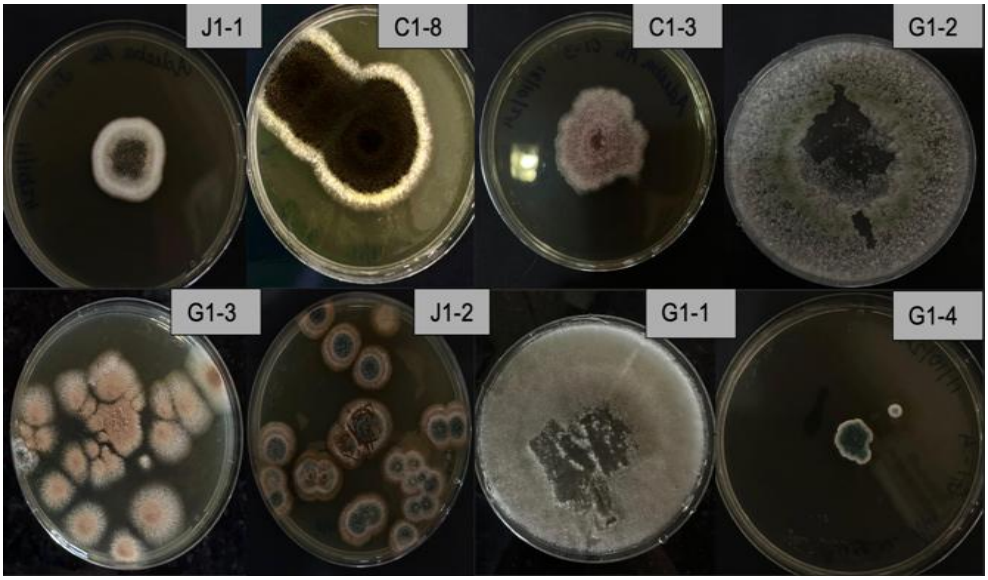

Figure 2: Isolated fungal strains grown on PDA plates

3.3 Primary screening

Starvation forces Fungi to degrade hydrocarbons (Figure 3) and helps researchers to understand metabolic pathways involved in breaking down PAHs and potential applications in bioremediation. Primary screening was carried out using BBH medium in order to evaluate the degradation of PAH by each fungal strain. BBH media contain minimal nutrients necessary for survival but lack carbon sources, which inhibits the growth of fungal strains and allows them to enter a state of stress-induced dormancy[15]. During the PAH spiked plate assay, sample G1-2 only showed a growth in Naphthalene and all the other sample grew on both Naphthalene and Phenanthrene spiked plates.

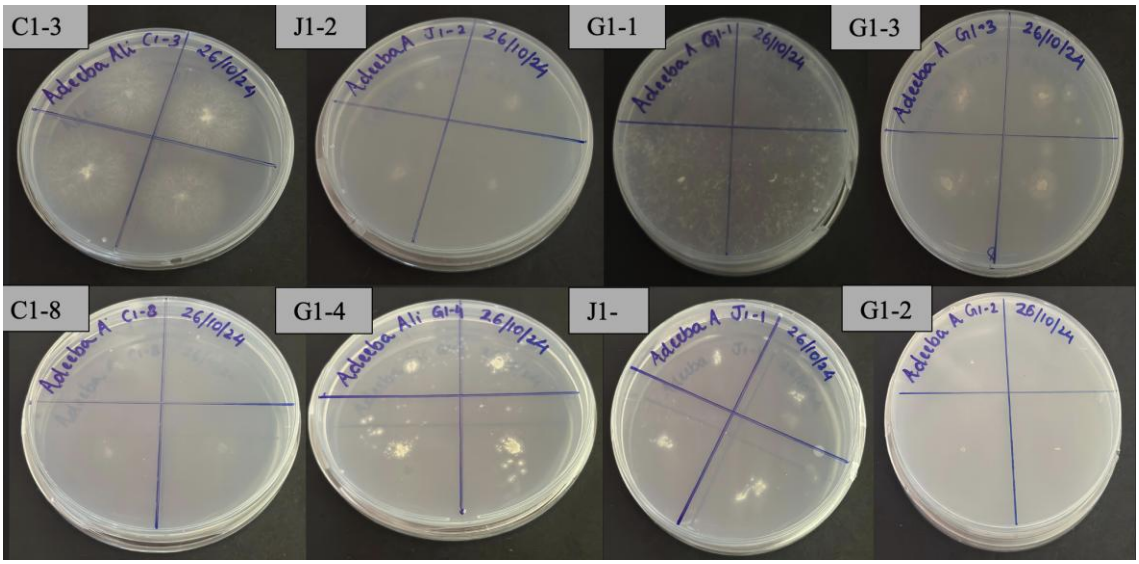

Figure 3: Primary screening of isolates from Galle (G1), Jaffna (J1) and Colombo (C1). The samples were incubated leading to fungal growth on plates.

Their observed growth illustrates the potential application of these fungal strains in PAH degradation. This highlights their adaptive response and enzymatic degradation. The results indicate maximum growth of fungal strains indicating their ability to utilize PAHs efficiently. Growth differences between the two PAHs indicates that the fungal strains may have distinct metabolic pathways or substrate preferences[16].

3.4 Molecular Analysis

C1-3 was identified as *Fussarium verticillioides* isolate with accession number of PV225534. It is a plant pathogen affecting mainly maize and it belongs to the section *Liseola* of the fusarium genus and phylogenetically defined as a member of the *Fusarium fujikuroi* species complex[17]. G1-1 was identified to be *Trichoderma harzianum* isolate with an accession number PV225528. It belongs to genus *Trichoderma* and is used as a bioagent used as a fungicide and also promotes plant growth. JI-2 was identified to be *Talaromyces verruculosus* with accession number PV225531. It belongs to the genus *Talaromyces.*

3.5 Kinetic Analysis

In this research, the half-life of fungi is the time taken by half of the fungal population to degrade the PAHs. In order to break down recalcitrant pollutants fungi primarily use cytochrome P450 monooxygenases and extracellular enzyme system that includes manganese and lignin peroxidases [18]. A shorter half-life indicates the fungi's ability to break down PAHs more quickly and efficiently. For naphthalene, out of the three fungal strains, C1-3 (*Fusarium verticillioides*) has a better degrading activity due to its lower half-life compared to the other two strains. Although previous studies have not reported half- life values of *Fusarium* species, their findings on higher degradation percentage support the observed rapid degradation by *Fusarium verticillioides* in the present study. Given the remarkable effectiveness in breaking down 1% naphthalene, one study also indicated that *Fusarium* species have a strong capacity of degrading PAHs. *Fusarium* demonstrated upto 98% degradation over 9 days using methylene blue assay[19]. The same applies for PHE, C1-3 appeared to have a shorter half-life compared to other two strains resulting in a quick degrading activity. This in line with earlier research that demonstrated the high phenanthrene breakdown efficiency of *Fusarium solani* as vertified by HPLC analysis, underscoring their significance potential for mycoremediation in PAH contaminated environments[20].Therefore, the fungal strain C1-3 with the shortest half-life is most efficient in degrading PAHs compared to the other two strains J1-2 and G1-19 (table 1).

Table 1: Half-life values of different fungal strains during the degradation of both PAHs. The values represent the time taken for 50% of degradation of each PAH by the respective fungal strains.

Sample	Half-life	
	NAP	PHE
Fusarium verticillioides	2.855 $days^{-1}$	3.215 $days^{-1}$
Trichoderma harzianum	2.253 $days^{-1}$	2.186 $days^{-1}$
Talaromyces verruculosus	4.194 $days^{-1}$	4.194 $days^{-1}$

3.6 Statistical Analysis

The results showed significant differences ($p<0.05$) in the degradation of PHE among the three fungal strains (figure 6). Since, the p-value is extremely lower, the null hypothesis is rejected indicating that at least one of the fungal strains has a significantly different degrading rate compared to the other two strains. This might be due to production of higher levels of lignolytic enzymes, a more efficient metabolic pathway to degrade PAHs and rapid fungal growth.

3.7 Degradation rate of PAH

However, during the NAP degradation, the p value appeared to be 0.59056 indicating no significant differences ($p>0.05$) in the degradation of NAP among the three fungal strains. Since, the p-value is extremely high, the null hypothesis is accepted indicating that the lack of variation in degradation rates could be due to production of similar levels of lignolytic enzymes and metabolic capacities and also the strains could be substrate specific.

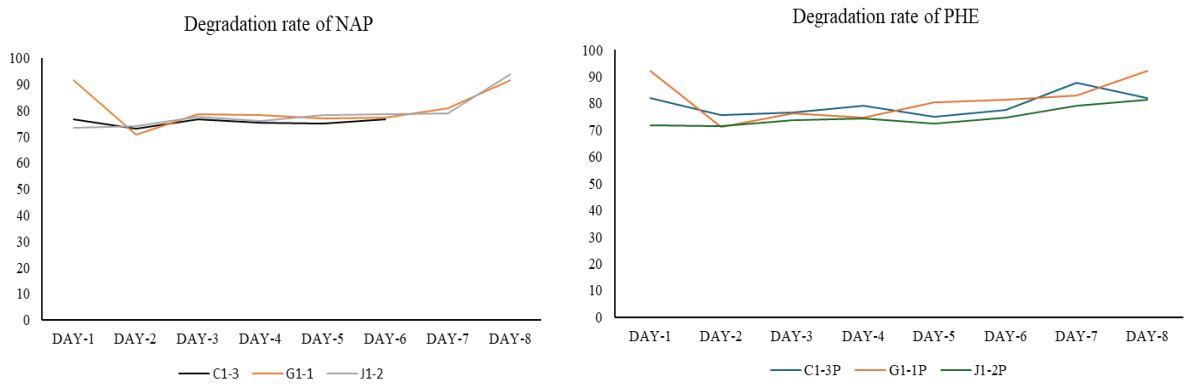

Figure 4: Degradation rate of NAP and PHE over the course of 8 days, expressed in terms of percentage.

The graph (figure 4) illustrates the degradation rate of NAP and PHE over a course of 8 days by three fungal isolates, i.e., C1-3: PV225534 *Fusarium verticillioides,* G1-1: PV225528 *Trichoderma harzianum* and JI-2: PV225531 *Talaromyces verruculosus.*

The following degradation pattern was observed. C1-3 and G1-1 showed a non-linear pattern in both the graphs (figure 8) suggesting the production of secondary metabolites or delayed production of PAHs degrading enzymes. The secondary metabolites might interfere with the degradation of PAHs and either slow or speed up the degradation rate. J1-2 suggest a linear degradation pattern with consistent degradation with minimal fluctuations and stable degradation in both the graphs (figure 4). All three stains are effective biodegradation agents as they have degradation percentage above 70% in both graphs (figure 8). However, in the case of NAP J1-2 is the most effective stain as its' degradation percentage is more than 90% whereas in the case of PHE, G1-1 is the most effective stain as its' degradation percentage is more than 90%. In, Gao *et al* 2019., reported *Talaromyces verruculosus* DJTU-SJ5 strain isolated from rhizosphere soil of *Taxus mairei* showed higher degradation of PAHs than other strains.[21] A study also reported that *Pleurotus ostreatus* and *Irpex lacteus* degraded 65%-80% of PHE out of 400 mg kg^{-1} after 28 days of incubation and *Trichoderma asperellum* could degrade 74% of PHE out of 1000 mg kg^{-1} after 14 days of incubation[22,23]. This indicates that the *Trichoderma* species have a better degrading ability than the white rot fungi which are said to be excellent degraders of PAHs.

3.8 Toxicity Assay

During the first hour, both NAP and PHE showed no mortality, suggesting that both do not have immediate lethal effects. During the 2^{nd} to 4^{th} hour in PHE there is an increase in the mortality rate in all three strains whereas there is a significant decrease of nauplii in C1-3. In NAP, for J1-2 and G1-1 the mortality rate stays the same during 2^{nd} to 4^{th} hour indicating NAP doesn't significantly affect mortality during this short-term exposure, whereas for G1-1 there is a slight increase in mortality rate. After 24 hours, for PHE in C1-3 there is a significant increase in mortality rate reaching to 2 nauplii whereas in J1-2 and G1-, there is a slight increase in mortality rate. For NAP, after 24 hours there is a slight increase in mortality rate for all three strains, this indicates that NAP causes lower mortality rate compared to PHE after 24 hours. The final observation was taken after 48 hours, for PHE in C1-3 the mortality rate reached 0 indicating that the compound is very toxic. PHE has a significantly higher level of toxicity due to a higher level of mortality rate particularly in C1-3. On the contrary, NAP has a lower level of toxicity due to gradual increase in mortality rate over the course of 48 hours. C1-3 is more sensitive to pollutants as it has a more significant increase in mortality rate.

Table 2: Viability of brine shrimp nauplii (*Artemia salina*) exposed to PHE, NAP and fungal degraded by-products over time.

Time	C1-3		J1-2		G1-1	
	PHE	NAP	PHE	NAP	PHE	NAP
1st hour	10	10	10	10	10	10
2nd hour	8	10	10	10	10	10
3rd hour	7	9	10	10	10	9
4th hour	6	9	9	9	10	9
24 hours	2	7	8	8	10	8
48 hours	0	6	7	7	10	7

3.9 Phylogenetic tree

C1-3 and G1-1 share a common ancestor whereas J1-2 has an ancestor that does not share a common ancestor with C1-3 and G1-1 (figure 5). According to previous researches *Trichoderma* and *Fusarium* which are members of the class Sordariomycetes order Hypocreales, have a same evolutionary origin, indicating their close taxonomic relationship within fungal lineage[24]. Although both of them share the common ancestor, studies have shown that *Talormyces* is in within the phylum Ascomycota and class of Eurotiomycetes, proving that it evolved from another ancestor[25]. *Trichoderma lentiforme voucher* has a totally different ancestor which is not common to any of the other three fungal strains obtained from NCBI GenBank. Although, Fusarium *verticillioides* and *Trichoderma harzianum* are different species they share a common ancestor but *Trichoderma lentiforme voucher* and *Trichoderma harzianum* being the same species do not share a common ancestor.

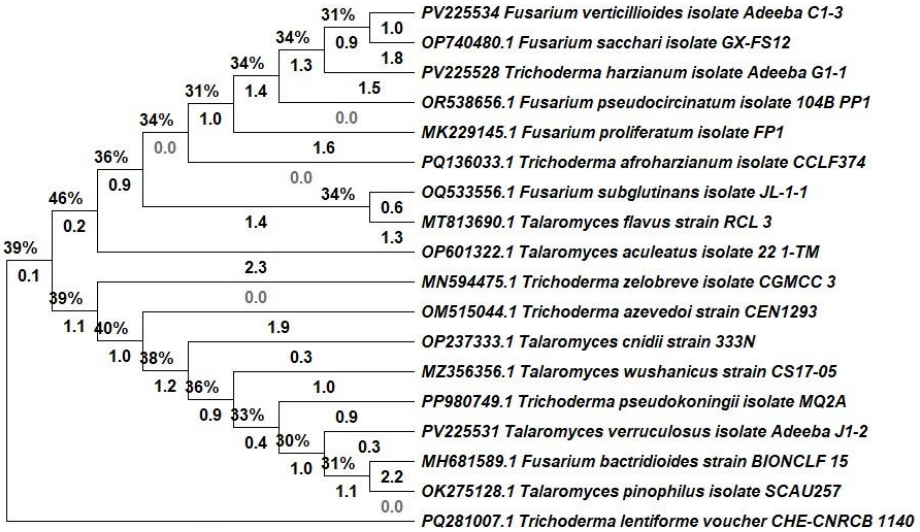

Figure 5: Phylogenetic tree illustrating the evolutionary relationship among the three fungal strain

4. Conclusion

The current study highlighted the biodegradation abilities of three different fungal species, i.e., *Fusarium verticillioides* PV225534, *Trichoderma harzianum* PV225528 and *Talaromyces verruculosus* PV225531 isolated from contaminated soil samples collected from three different locations across the country. The fungal strain C1-3, *Fussarium verticillioides* PV225534 was assumed as the most efficient strain in degrading PAHs compared to the other two strains J1-2 and G1-1 due to its shortest half-life. However, *Talaromyces verruculosus* PV225531 showed the best degradation capability in the case of NAP as the degradation percentage is more than 90% whereas *Trichoderma harzianum* PV225528 showed the best degradation

capability in the case of PHE as it's degradation percentage above 90%. The toxicity count revealed C1-3 being more sensitive to the pollutant as it has a more significant increase in mortality rate. According to the findings in this research fungal strains such as *Talaromyces verruculosus* PV225531 and *Trichoderma harzianum* PV225528 can be used as bioremediation agents in the future. These fungal strains can be incorporated into a biofilter to degrade and remove contaminants such as PAHs from air, water or soil aiding in pollution control making them agents in bioremediation.

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