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ASSESING DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN FRONT OF TIN (Sn) STRESS BY A SOIL BACTERIAL CONSORTIUM

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Polycyclic Aromatic Hydrocarbons (PAHs) are persistent in the environment and are toxic pollutants which have carcinogenic and mutagenic properties. They are composed of two or more fused benzene rings. Bacteria present in soil are capable of degrading these compounds via anaerobic and aerobic respiration. However, heavy metals such as tin have the ability to hinder the degradation capabilities of bacteria. The goal of this study is to isolate and identify soil bacteria capable of degrading Naphthalene and Phenanthrene in the presence of heavy metals. Soil samples were collected from 4 different locations in Sri Lanka: Colombo, Galle and Jaffna, as they are highly polluted areas according to previous literature and Meemure, as it had the lowest PAH pollution according to studies and were serially diluted and plated. Morphologically different strains were identified and starved on Bushnell-Haas Agar. Primary screening was performed using Naphthalene and phenanthrene spiked plates. Five bacterial strains: RUSHJ08, RUSHC09, RUSHMO6, RUSHG02, and RUSHG04, were able to degrade these PAHs were identified and were a subjected to second screening using methyl blue. UV-Spectrophotometric analysis revealed that these bacteria can degrade over 40% of the PAHs under optimum conditions. RJO8 (Accession number PV942241) and RJO2 (Accession number PV942264) were identified as best PAH degraders. Gram staining was performed to identify Gram-negative and Gram-positive bacteria, and antagonistic assay was performed to identify the capability of the strains to exist as a consortium. Atomic Absorption Spectroscopy was performed to evaluate the presence of heavy metals in the Colombo soil sample, and the concentration of tin was 2.22mg/kg. The results of this experiment support the development of a bioremediation technique in tin-stressed environments, which provides a solution to environmental pollution.

Keywords: Phenanthrene, Naphthalene, heavy metals, spectrophotometric, bioremediation.

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INTRODUCTION

PAHs are a group of organic compounds made of multiple fused aromatic rings and are famously known for being carcinogenic and common environmental pollutants usually found in petroleum coal and smoke (Lawal, 2017). They are hydrophobic substances which are a by-products of incomplete combustion. Their structure is composed of 2 or more interconnected benzene rings. Some of the simplest types of PAHs are Phenanthrene and Naphthalene. PAHs have the ability to attach to DNA which results in genetic mutations and leads to greater cancer risk. As they are significant environmental pollutants, it is vital to study the methods of degradation (Elufisan et al.,2024). PAHs can also accumulate in soil, water, and air, which makes them long-term contaminants in the environment. Different bacterial species have the ability to degrade via aerobic and anaerobic pathways through enzymatic oxidation and further degrade it in metabolic cycles. *Pseudomonas* and *Rhodococcus* are identified PAH degraders which break them down via oxidation. Tin(Sn), a heavy metal has the ability to hinder PAH degradation as it can exist as organotin compounds such as tributyltin in the environment which can disrupt enzyme function by binding to oxygenase's (Czarny et al., 2020). It is pivotal in identifying a method to degrade PAHS in co-contaminated environments involving the presence of heavy metals.

METHODOLOGY

Soil samples were collected from 4 different locations in Sri Lanka: Galle, Jaffna, Meemure and Colombo. Galle, Jaffna, and Colombo were selected and previous literature proved that these are highly polluted locations due to high urbanization. Meemure was selected as the control site as it is known to have the lowest contamination of PAHS. The samples were serially diluted using NaCl and 10^{-4} and 10^{-8} were plated on nutrient agar plates using the spread technique. Ten morphologically different bacterial strains were selected and were plated using the streak plate technique. These strains were starved for 3 days on Bushnell-Haas (BBH) agar to eliminate any stored carbon. Subsequently, primary screening was performed using Naphthalene and Phenanthrene spiked plates to identify PAH degraders, Secondary screening was then performed using BBH broth and methyl blue was used to identify the degradation of the PAHS via spectrophotometry. The experiment was divided into two sets, where phenanthrene degradation was observed separately and similarly for phenanthrene and was performed in triplicate. It was left for 7 days and the best degraders were identified by

calculating degradation percentages. Gram staining was then performed to identify Gram-negative and Gram-positive bacteria and to identify their morphologies. An antagonistic assay was carried out to analyse the ability of the strains to coexist as a consortium without inhibiting each other. Atomic Absorption Spectroscopy (AAS) was performed to identify the presence of heavy metals in the soil samples.

RESULTS AND DISCUSSION

The serially diluted samples were plated via the spread plate technique and 10 morphologically different bacterial strains were selected to proceed as seen in Figure 1.

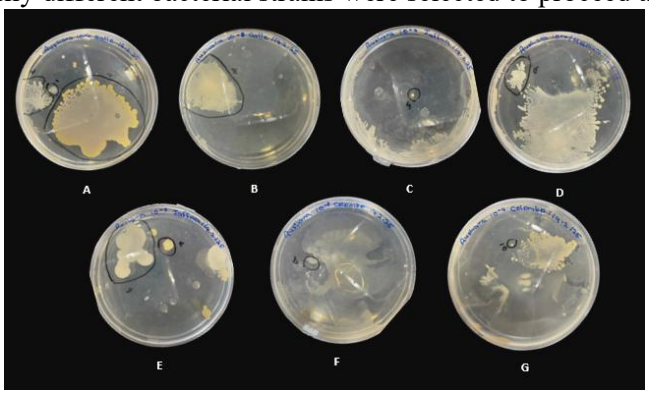

Figure 5: - Spread plate assay of diluted soil samples: A-Galle 10-4, B- Galle 10-8, C-Jaffna 10-4, D-Meemure 10-4,E -Jaffna 10-8,F-Colombo 10-8, G-Colombo 10-4

These strains were then further isolated using the streak plate techniques as seen in Figure 2. This allowed further dilution of strains to separate colonies, further segregation of colonies, and isolation of pure cultures. Sample J shows two different bacterial strains growing which were not identified in the spread plate (Wang et al., 2021; Zhou et al., 2023).

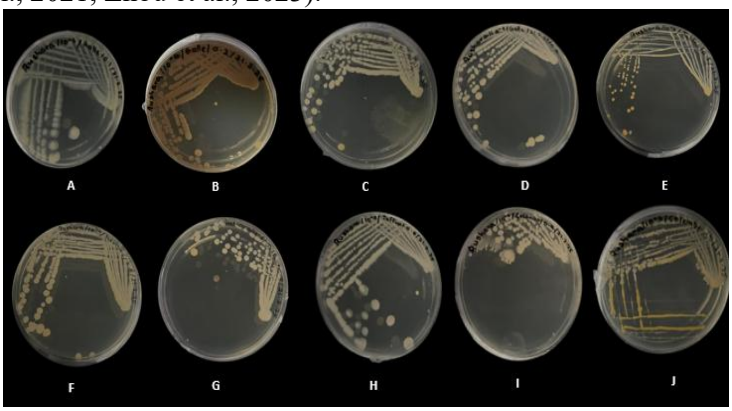

Figure 6:- Streak plate assay of bacterial strains: A- RUSHG01, B- RUSHG02, C- RUSHG03, D- RUSHG04, E- RUSHJ05, F- RUSHM06, G- RUSHJ07, H- RUSHJ08, I- RUSHC09, J- RUSHC10.

The strains were then starved on BBH agar as it lacks a carbon source creating a nutrient-limited environment so that stored carbon was used and only PAH degrading bacteria would survive in the primary screening step as seen in Figure 3.

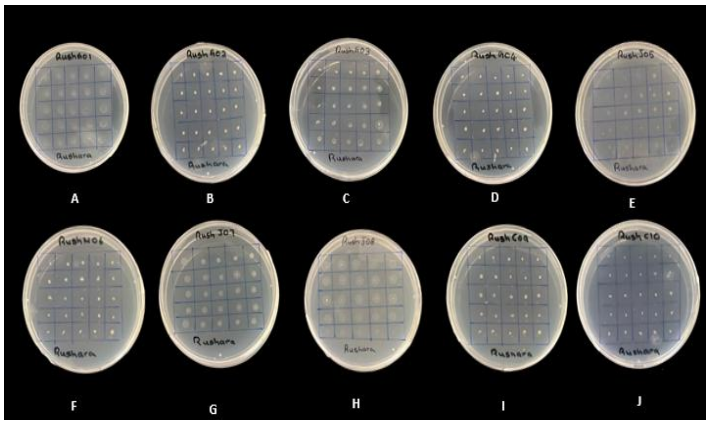

Figure 7 :- Starvation of bacteria on BBH agar: A- RUSHG01, B- RUSHG02, C- RUSHG03, D- RUSHG04, E- RUSHJ05, F- RUSHM06, G- RUSHJ07, H- RUSHJ08, I- RUSHC09, J- RUSHC10

Primary screening was performed using Naphthalene and phenanthrene spiked plates and growth of the colonies was observed in order to identify strains with degradation capabilities. It was observed that 5 strains were able to degrade these 2 PAHS, whereas strains such as RUSHJ07 was only capable of degrading Phenanthrene as seen in Figure 4.

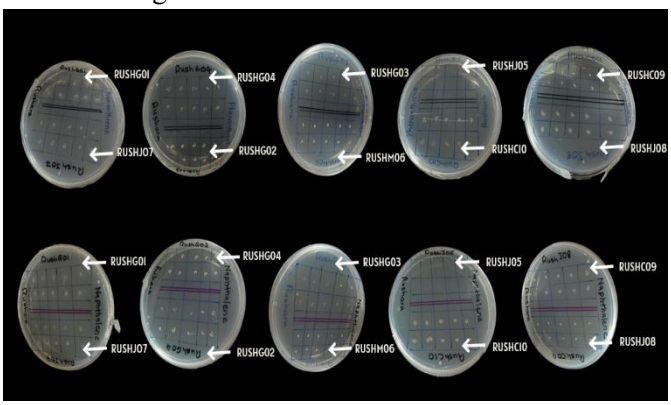

Figure 8: Primary screening of bacteria on spiked plate with Naphthalene and Phenanthrene

The growth of colonies of the strains capable of degradation is seen in Table 1.

Table 4:- Summary of growth of bacteria on naphthalene and phenanthrene-spiked plates

Sample Code	Phenanthrene	Colony Count	Naphthalene	Colony Count
RUSHC10	Growth observed	7/10	Growth observed	10/10
RUSHJ05	No Growth	0/10	No Growth	0/10
RUSHM06	Growth observed	10/10	Growth observed	10/10
RUSHG03	Minimal growth	2/10	Minimal growth	2/10
RUSHC09	Growth observed	10/10	Growth observed	10/10
RUSHJ08	Growth observed	10/10	Growth observed	10/10
RUSHG01	No Growth	0/10	No Growth	0/10
RUSHJ07	Growth observed	10/10	Minimal growth	4/10
RUSHG02	Growth observed	10/10	Growth observed	10/10
RUSHG04	Growth observed	10/10	Growth observed	6/10

Strains RUSHC09, RUSHJ08, RUSHM06, RUSHG04, and RUSHG02 were identified as best degraders and were selected to proceed with secondary screening as they had the highest growth of colonies in both PAHS.

Secondary screening was performed using spectrophotometry to analyse the reduction of methylene blue to leucomethylene which is a colourless compound signalling degradation of PAHS. Degradation percentages were calculated and RUSHJ08 was identified as the best degrader as seen in Figure 5. This aligns with previous findings by Wang et al. (2021) proving that degradation of PAHs is a reducing reaction and uses the enzyme dioxygenase. The reduction of methylene blue proved that PAH Degradation occurred. Strains RJ08 (Bacillus cereus) and RG02 (Bacillus megaterium) were sequenced and confirmed by Gram staining, with accession numbers submitted to GenBank. RJ08 (Accession number PV942241), isolated from Jaffna soil, and RG02 (Accession number PV942264), from Galle soil, demonstrated strong PAH-degrading abilities consistent with previous studies, highlighting their significance in polluted environments. (Das, Bhattacharya, Banu, & Kotoky, 2017

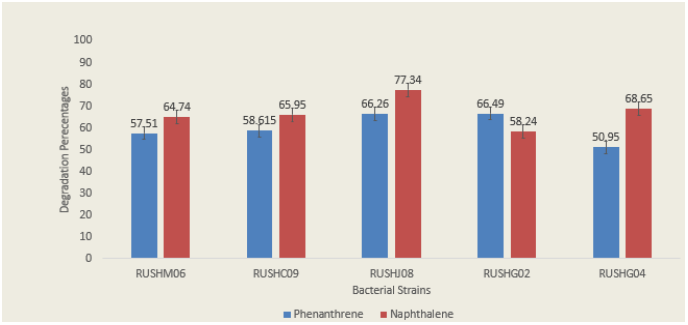

Figure 9- Degradation Percentages of different bacterial strains of phenanthrene and naphthalene

Gram staining was performed to identify Gram-negative and Gram-positive bacteria from the selected strains and morphologically different bacteria as seen in Figure 6.

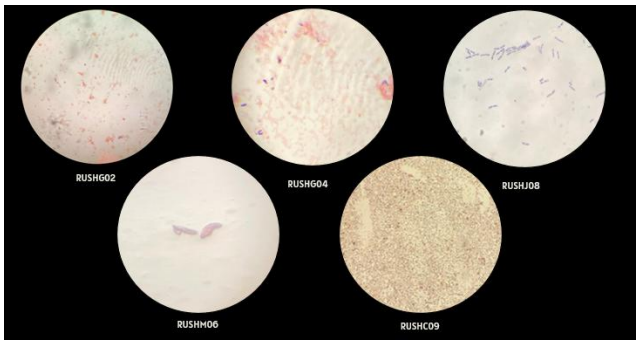

Figure 10- Results of gram staining for PAH degrading bacteria

The antagonistic assay was performed to evaluate the strains to exist as a consortium, and Figure 7 shows that they are able to co-exist as no inhibition or antagonistic effect was seen. The absence of an antagonistic effect supports the formation of a stable bacterial consortium which may also have the ability to enhance degradation through synergistic interactions (Aruotu et al., 2023).

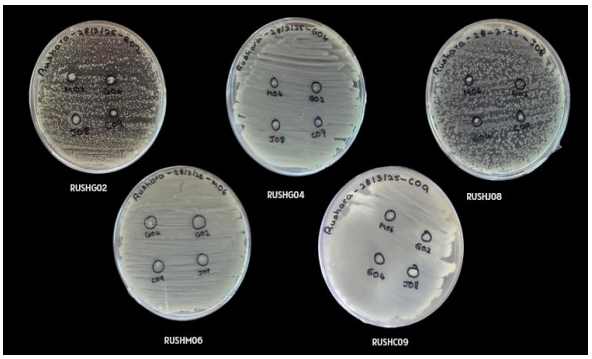

Figure 11:- Antagonistic assay on the bacterial strains

AAS confirmed the presence of Sn in the Colombo soil sample and the concentration percentage was 2.22mg/kg which is well below the human hazardous levels as seen in Figure 8.

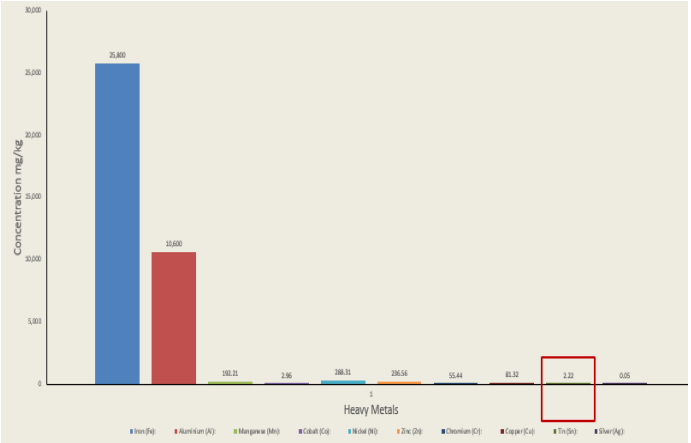

Figure 12- Presence of ten heavy metals in Colombo soil sample analyzed using atomic absorption spectroscopy

These findings support the potential use of a bacterial consortium in bioremediation of PAH-contaminated, metal-stressed environments.

CONCLUSIONS and RECOMMENDATIONS

This study demonstrates that selected soil bacteria possess dual capabilities: tolerance to tin and degradation of PAHs, making them strong candidates for bioremediation strategies. Future work should include optimization of degradation conditions in field-scale trials and genomic analysis to further characterize resistance and degradation pathways. The consortium approach may be extended to remediate other co-contaminated sites, contributing to cost-effective and sustainable environmental clean-up in alignment with the UN Sustainable Development Goals (SDGs).

This PAH degradation study of a soil bacterial community is pivotal in developing effective soil remediation strategies specially in harbours as PAH pollutants and heavy metals are abundantly found. Future work, includes assessing degradation capabilities of the consortia in from of a range of tin concentrations, DNA extraction, PCR, Gel electrophoresis and sequencing to identify these strains and additionally a toxicity assay to evaluate the toxicity of by-products produced by this reaction.

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