Abstract

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are a group of hazardous and persistent organic pollutants that pose serious threats to both the environment and human health. Their widespread distribution is mostly from industrial activities, automobile emissions, and incomplete burning of organic materials. As derivatives of polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs are characterized by their high toxicity, which leads to endocrine and neurological system disruptions, oxidative stress, DNA damage, and mutagenic and carcinogenic effects. Furthermore, their phytotoxic effects interfere with plant growth and development, and their accumulation in plants and biomagnification along the food chain exacerbate their environmental risks. Nitro-PAH contamination is a serious problem and hence, effective remediation solutions are warranted for restoring polluted ecosystems and protecting life on land for a sustainable future. Therefore, this study explores a green and sustainable approach to eliminating two types of nitro-PAHs using plant-associated microbes and biostimulants.

This study investigated the synergistic effect of microbial co-inoculum and biostimulant application on the phytoremediation of nitro-PAHs, especially 1-nitropyrene and 2-nitrofluorene. Four plant species-*Cyperus rotundus* L., *Cyperus esculentus* L., *Axonopus compressus* (Sw.) P. Beauv., and *Imperata cylindrica* (L.) Raeusch. -were selected based on their high Importance Value Index (IVI) at the sample location in Borholla (26°45' latitude and 94°37' longitude). *C. rotundus* had the highest IVI (25.55) of the 15 identified plant species, followed by *A. compressus* (25.50), *C. esculentus* (25.00), and *I. cylindrica* (19.77). Over a 60-day incubation period, the selected plant species underwent evaluation for their ability to accumulate 1-nitropyrene and 2-nitrofluorene inside root tissues. Among these species, *I. cylindrica* had the highest accumulation potential reaching 250 mg/g for 1-nitropyrene and 279.6 mg/g for 2-nitrofluorene. *A. compressus* also accumulated significantly, with 220 mg/g of 1-nitropyrene and 270.8 mg/g of 2-nitrofluorene, whereas *C. rotundus* and *C. esculentus* accumulated 210 and 200 mg/g of 1-nitropyrene and 240 and 233.8 mg/g of 2-nitrofluorene. Two rhizobacterial strains, *Bacillus cereus* BG034, and *Bacillus altitudinis* BG05, were isolated from the root zone of these plant species. Further study has been conducted to see their tolerance to nitro-PAHs and plant growth-promoting traits, which included phosphate solubilization, siderophore, ammonia and hydrogen cyanide (HCN) production, indole acetic acid (IAA) synthesis, sucrose fermentation. A bacterial co-inoculum (BGC01) was formulated by combining these isolated rhizobacteria. The nitro-PAHs degradation potential of the isolates

and their co-inoculum (BGC01) were evaluated, with *Bacillus cereus* BG034 achieving 47.84% degradation of 1-nitropyrene and 59.96% of 2-nitrofluorene within 72 hours. Similarly, *Bacillus altitudinis* BG05 degraded 49.04% of 1-nitropyrene and 59.88% of 2-nitrofluorene.

The formulated bacterial co-inoculum (BGC01) exhibited significantly higher degradation efficacy, achieving 76% and 87.20% degradation of 1-nitropyrene and 2-nitrofluorene, respectively. The kinetic study demonstrated that the degradation of 1-nitropyrene followed a second-order kinetic decay model, while the degradation of 2-nitrofluorene followed a first-order kinetic decay model. The co-inoculum BGC01 had the highest rate constant (k = 0.0018 L mol^{-1} h^{-1}) and the lowest half-life ($T_{1/2}$ = 22.22 hours) for 1-nitropyrene degradation, compared to the separate strains *Bacillus cereus* BG034 and *Bacillus altitudinis* BG05 (k = 0.0005 L mol^{-1} h^{-1}, $T_{1/2}$ = 80 hours). The co-inoculum (BGC01) also had a higher rate constant (k = 0.0284 L mol^{-1} h^{-1}) and shorter half-life ($T_{1/2}$ = 24.40 hours) than *Bacillus cereus* BG034 (k = 0.0122 L mol^{-1} h^{-1}, $T_{1/2}$ = 56.80 hours) and *Bacillus altitudinis* BG05 (k = 0.0124 L mol^{-1} h^{-1}, $T_{1/2}$ = 55.89 hours) for 2-nitrofluorene breakdown. Moreover, greenhouse experiments demonstrated that the formulated bacterial co-inoculum (BGC01) significantly enhanced plant growth compared to individual strains.

In this study, a biostimulant was formulated which was rich in essential amino acids and also demonstrated plant growth-promoting properties. When it was applied together, the plant-bacterial co-inoculum (BGCP01) and biostimulant significantly improved plant growth by alleviating oxidative stress induced by nitro-PAHs by increasing enzymatic activities like superoxide dismutase, ascorbate peroxidase, catalase, and peroxidase. Furthermore, a microcosm study was also carried out to see the degradation pathways of nitro-PAHs, revealing a stepwise breakdown of 1-nitropyrene and 2-nitrofluorene aided by bacterial enzymatic activities and assisted by plant-mediated detoxification processes. The plant-bacterial co-inoculum (BGCP01) catalyzed nitro-PAHs by nitro group reduction, hydroxylation, and ring fragmentation, which resulted in the production of less toxic intermediates. In this study it was revealed that the combined effect of plant and bacterial metabolic activity significantly enhanced biodegradation efficiency, finally converting complex aromatic compounds into simpler aliphatic hydrocarbons such as decane. Furthermore, the synergistic use of the plant-bacterial co-inoculum (BGCP01) and biostimulant resulted in significant improvements in soil physicochemical properties. Nitrogen, phosphorous, potassium, and organic carbon contents increased significantly, boosting soil fertility and promoting ecosystem regeneration. The ability of the designed inoculum to improve soil health while simultaneously increasing pollutant breakdown highlights its promise as a new bioremediation technique. This study highlighted microbial and biostimulant-assisted phytoremediation as an environmentally sustainable alternative for the degradation of nitro-PAH in soil.

This thesis was systematically structured to present a clear and logical progression of the research, ensuring a comprehensive understanding of the significance of the study, methodology, and findings. It was organized into five chapters, each of which addressed a specific aspect of the study.

- **Chapter I: Introduction** – Provides an overview of the research, including the problem statement, objectives, and significance of the study.
- **Chapter II: Literature Review** – Examines existing research on nitro-PAHs, phytoremediation, bacterial degradation, and biostimulants, establishing the study's foundation.
- **Chapter III: Methodology** – Details the experimental design, materials, and analytical techniques used to assess phytoremediation efficiency.
- **Chapter IV: Results and Discussion** – Presents and interprets research findings, supported by statistical analysis and comparisons with existing literature.
- **Chapter V: Conclusion and Future Prospects** – Summarizes key findings and suggests directions for further research.