Chapter I: Introduction

1.1. Study Background

The twentieth century witnessed an unprecedented wave of industrialization that brought about remarkable economic development and significantly improved living standards across the globe. However, as Anne Hope Jahren, an American geochemist and geobiologist, underscores in her landmark book *The Story of More*, this rapid progress has been accompanied by profound environmental challenges rooted in unsustainable human consumption patterns. Through her exploration of key innovations such as electric power, large-scale agriculture, and automobiles—Jahren highlights their dual role in advancing human society while simultaneously contributing to untenable pollutant emissions. Her work invites a critical reflection on how these advancements, while transformative, have exacerbated ecological degradation, thereby threatening the planet's resources. Within this broader context of environmental concerns, one particularly urgent issue has emerged: soil contamination from nitrated polycyclic aromatic hydrocarbons (nitro-PAHs), a byproduct of industrial processes with significant implications for both ecosystem health and human well-being.

Nitro-PAHs are derivatives of polycyclic aromatic hydrocarbons (PAHs) that are formed through a nitration process when PAHs interact with nitrogen oxides (NOx) (Bandowe & Meusel, 2017; Zhang et al., 2011). Natural sources, such as volcanic eruptions and forest fires, release PAHs which undergo a nitration process to form nitro-PAHs. However, anthropogenic activities, especially the burning of fossil fuels such as coal, natural gas, and petroleum, are the predominant contributor to the increasing concentration of nitro-PAHs in our environment (Huang et al., 2014; Venkatraman et al., 2024). Furthermore, nitro-PAHs can be generated from indirect and direct sources. When PAHs are exposed to sunlight, especially in the presence of nitrogen oxides, hydroxyl radicals, and nitrate radicals, a series of photochemical reactions are triggered (Jariyasopit et al., 2014). These radicals and oxides play essential roles in the photochemical process, facilitating the conversion of PAHs into nitro-PAHs (Lee et al., 2022). Nitro-PAHs possess several distinguishing features, including higher melting temperatures, octanol-air partition coefficients, octanol-water partition coefficients, organic carbon-water partition coefficients, low vapor pressure, and particle-gas partition coefficients (Bandowe & Meusel, 2017). These pollutants can travel long distances through air currents, water bodies, and soil due to their above-mentioned characteristics (Qi et al., 2023; Yang et al., 2020). Due to their low water solubility, nitro-PAHs are transported to deeper soil layers through colloid-assisted transport (Cao et al., 2022).

Soil serves as a major sink for nitro-PAHs. The highest concentrations of nitro-PAHs are typically found in the topsoil, with levels decreasing as soil depth increases due to their hydrophobic nature (Bandowe & Meusel, 2017). Their strong hydrophobicity and persistent nature adversely affect several environmental variables, including the soil microbial communities, plants, aquatic organisms, and humans (Li et al., 2022; Menezes et al., 2023).

Nitro-PAHs have also been reported to cause severe effects in aquatic organisms, terrestrial organisms, plants, and microorganisms, including humans, by inducing oxidative stress, genotoxicity, and metabolic disruptions. Increased nitro-PAH concentrations in soil altered microbial community, reduced diversity, and disrupted metabolic functions (Anyanwu & Semple, 2018). Nitro-PAH exposure caused selective pressures on nitro-PAH-resistant microbes by promoting their growth and development process while suppressing those that are more sensitive, resulting in disrupted microbial balance in the soil. Furthermore, high nitro-PAH concentrations impair their enzyme functioning, impede breakdown processes, and obstruct critical metabolic pathways, reducing microbial viability. Excessive amounts also limit the growth of degradative microbes, prolong lag times, and reduce CO_2 generation (Anyanwu & Semple, 2016; Zhu et al., 2019). Similarly, in plants, nitro-PAHs impose significant toxicity, by affecting growth, physiological processes, and metabolic activities. Upon root uptake, these pollutants generate reactive oxygen species (ROS), which cause oxidative stress, lipid peroxidation, and membrane damage (Yun et al., 2019). Prolonged exposure further inhibited seed germination, stunted growth, reduced biomass, and impaired photosynthesis due to chlorophyll degradation (Alp-Turgut et al., 2024). Furthermore, nitro-PAHs have genotoxic effects, including DNA damage, chromosomal abnormalities, and mutations that impair plant growth and development (Yang et al., 2024). Despite these challenges, several hyperaccumulator species show resistance by sequestering nitro-PAHs within root above-ground tissues and activating enzymatic detoxification pathways, therefore decreasing ROS-induced damage and assisting phytoremediation efforts in polluted environments. Hence, mitigating nitro-PAH pollution is a global concern.

The United Nations Sustainable Development Goals (SDGs) were adopted by the United Nations (UN) in 2015 for the period 2016–2030, comprising 17 goals and 169 targets aimed at creating a habitable world for living organisms through pollution reduction and the sustainable use of natural resources (Aslam et al., 2024). Among these, pollution poses a significant threat to achieving SDG 14, which focuses on life below water. This is because pollutants, including emerging contaminants, run off into water bodies, contaminating water sources and threatening aquatic life. Exposure to such pollutants adversely impacts reproductive and developmental processes in aquatic organisms, leading to population declines and disruptions in aquatic ecological balance (Kong et al., 2023). Furthermore, these emerging contaminants can bioaccumulate in the tissues of aquatic organisms, impairing higher trophic levels and potentially affecting entire food chains (Onduka et al., 2012). Consumption of contaminated seafood has been linked to DNA damage, oxidative stress, and an increased risk of cancers, particularly affecting the liver and gastrointestinal systems in humans, posing a direct threat to achieving SDG 3–good health and well-being.

Similarly, achieving SDG 6–clean water and sanitation for all–is increasingly challenging due to the detection of various emerging contaminants, including nitro-PAHs, in water sources over the past few decades. SDG 15, which focuses on protecting life on land, is also jeopardized as these pollutants adversely affect terrestrial ecosystems and soil health. Therefore, robust regulatory frameworks and mitigation strategies at local, regional, and global levels are essential to minimize the impact of emerging contaminants and support the achievement of these interconnected SDGs (Fig.1.1).

Fig. 1.1. Nitro-PAH pollution negatively impacts various environmental and health-related Sustainable Development Goals (SDGs). Specifically, it demonstrates how nitro-PAHs contribute to ecosystem degradation in both aquatic and terrestrial environments, deteriorate water quality, and pose serious risks to human health. These adverse effects hinder the achievement of SDG 3 (Good Health and Well-being), SDG 6 (Clean Water and Sanitation), SDG 14 (Life Below Water), and SDG 15 (Life on Land), emphasizing the need for effective pollution control and sustainable environmental management strategies.

The United States Energy Information Administration (EIA) forecasts that coal, oil, and natural gas will continue to account for 77% of worldwide energy demand by 2040, with petroleum consumption expected to rise from 95 million barrels per day (b/d) in 2015 to 113 million b/d by 2040 (EIA, 2025). Even though the global use of alternative green energy with zero emissions is gaining importance, reliance on fossil fuels to meet global energy demands remains inevitable in the current scenario. Despite efforts to promote renewable energy sources, fossil fuels continue to dominate the energy landscape. This substantial dependence on fossil fuels has significantly contributed to environmental pollution through the emission of 16 EPA (United States Environmental Protection Agency)-listed PAHs, primarily due to oil refining, combustion, and accidental spills (Bandowe & Meusel, 2017; Drotikova et al., 2021). As the world's third-largest oil consumer, India relies on imports to meet more than 85% of its crude oil needs (Amuda et al., 2023; Nazir & Rehman, 2021). With continued fossil fuel consumption, emissions of PAHs and their subsequent conversion into nitro-PAHs are expected to increase (Singh et al., 2020). Highly populated Indian cities such as Delhi, Mumbai, and Kolkata have recorded elevated levels of PAHs, primarily attributed to vehicular emissions and industrial effluents (Kaushal et al., 2021; Saha et al., 2017).

Assam has a major crude oil reserve and is the country's third-largest producer, behind Rajasthan and Gujarat. The history of oil exploration in Assam began with the discovery of oil fields at Digboi in 1889 (Bhagobaty, 2020). The Assam Oil Company, founded in 1899, played a crucial role in overseeing oil production activities (Bhattacharyya, 2022). Over the years, Upper Assam has unveiled more than 100 oil fields, including Naharkatiya, Moran, Hugrijan, Amguri, Rudrasagar, Geleki, Lakowa, and Borholla (Sarma et al., 2016). Five major corporations—Oil India Limited (OIL), Oil and Natural Gas Corporation Limited (ONGC), Indian Oil Corporation Limited (IOCL), Bharat Petroleum Corporation Ltd (BPCL), and Assam Hydrocarbon and Energy Company Limited (AHECL)—are actively involved in oil exploration and production in these areas. Accidental oil spills during crude oil drilling and transportation in these oil-producing areas require immediate attention (Sarma & Prasad, 2024). Oil extraction from different fields is gathered at the Group Gathering Stations (GGS), from which pipelines transport the oil to the downstream processing (Patowary et al., 2023). However, accidental spills (Fig. 1.2) during typical oil field activities lead to the unintended release of oil into the environment (Sarma et al., 2016). These spills are reported to contain PAHs that are further converted into nitro-PAHs through the nitration process. This contamination results in compromised soil fertility and water quality, which are critical for cultivating food crops. The proximity of Assam's oil fields to human settlements creates a unique cohabitation that raises significant environmental and health concerns (Sarma & Prasad, 2024). The relationship between petroleum industry activities and the agricultural soil in the region demonstrates severe environmental and social impacts, particularly in areas where rice fields and tea gardens flourish (Sharma et al., 2018). The presence of harmful substances, such as PAHs, in the environment can directly affect the health of residents and workers in these areas, complicating the relationship between industrial activities and public well-being (Sarma & Prasad, 2024).

Fig. 1.2. Oil spills in a paddy field at the study site—a common issue in Assam's oil fields. Crude oil infiltrates the soil, contaminating crops and ecosystems. EPA-listed PAHs further degrade into toxic nitro-PAHs, worsening environmental and health risks.

Given the harmful effects of nitro-PAHs on the environment, the need for effective mitigation strategies has become crucial. Various remediation methods, including chemical, biological, and physical approaches, have been developed to minimize the impact of oil spills (Falciglia et al., 2016; Tiwari et al., 2019). However, each of these methods presents specific limitations. For instance, although chemical remediation is highly effective in converting nitro-PAHs into less hazardous substances, it is often prohibitively expensive and not feasible for large-scale field applications (Chen et al., 2022; Falciglia et al., 2016). Physical remediation methods, which involve the mechanical removal of contaminants from the environment, are labor-intensive and require substantial time and effort (Ahmed et al., 2021; Ossai et al., 2020). Consequently, the development of more efficient and cost-effective remediation strategies remains a pressing challenge for environmental management and pollution control. However, biological remediation is an eco-friendly approach that utilizes living organisms to degrade or immobilize nitro-PAHs (Kumar & Shukla, 2024; Li et al., 2020). While this method is time-intensive, it is considered sustainable as it does not involve toxic chemicals or solvents (Kaya et al., 2024). The primary challenge lies in maintaining optimal abiotic conditions, such as adequate nutrient availability and the survival of the desired microbial population at the application site.

Several cost-effective and sustainable technologies have been explored in recent decades to enhance the capacity and performance of phytoremediation for removing organic pollutants, including PAHs, using beneficial bacteria and plants (Sarma et al., 2024). While specific studies on nitro-PAH phytoremediation are limited, this study has been designed to address this gap.

Plants possess a range of cellular mechanisms to remediate PAHs through phytoextraction, phytostabilization, phytovolatilization, and rhizoremediation (Manoharan & Veeraragavan, 2024; Rolón-Cárdenas & Hernández-Morales, 2024). Additionally, plants have complex enzymatic processes involved in the metabolism of organic pollutants. Enzymes such as cytochrome P450 monooxygenases and glutathione S-transferases play a crucial role in transforming these pollutants, contributing to the overall effectiveness of phytoremediation (Cheng et al., 2022; Kennes-Veiga et al., 2022).

Biostimulants are substances derived from biological sources that enhance plant growth and improve stress tolerance. These substances provide essential nutrients, supporting both plants and microbes in thriving under harsh environmental conditions during field applications (Bartucca et al., 2022; Magnabosco et al., 2023). Typically, biostimulants contain amino acids, minerals, and organic compounds, which help plant-associated microbes accelerate metabolism by acting as enrichment factors (Drobek et al., 2019; Udume et al., 2023).

The application of biostimulants has shown promising potential in phytoremediation due to their ability to enhance plant metabolic processes, enabling more effective detoxification of PAHs (Ali et al., 2023; Farruggia et al., 2024). Moreover, biostimulants stimulate microorganisms to produce enzymes that convert PAHs into less toxic metabolites. However, no studies have been identified so far on the application of biostimulant-assisted remediation for nitro-PAHs. Hence these experiments have been designed to explore the role of biostimulant and microbes to enhance the phytoremediation process to eliminate nitro-PAHs in both aerobic and anaerobic environments.

1.2. Scientific Limitation

Existing literature indicates that nitro-PAHs, particularly those originating from the petroleum industry, are emerging contaminants requiring urgent attention due to their persistent toxicity (Gogoi et al., 2025). However, an extensive literature search in databases such as Scopus, PubMed, and ScienceDirect did not yield any comprehensive studies specifically addressing the phytoremediation of nitro-PAHs. While some studies report the ability of certain bacterial species to degrade PAHs, no research has been found focusing on the phytoremediation of nitro-PAHs, leaving this area largely unexplored. Furthermore, the role of specific biostimulant, particularly in the phytoremediation of 1-nitropyrene and 2-nitrofluorene, remains unexamined.

1.3. Research Objectives

This study aims to enhance phytoremediation efficiency for nitro-PAHs by identifying key microbial strains and biostimulants. It focuses on optimizing their application to mitigate the persistence and environmental impact of 1-nitropyrene and 2-nitrofluorene.

The specific objectives are:

1.3.1. Isolation and Identification: Identify nitro-PAH-degrading rhizobacteria and nitro-PAH-accumulating plants.

1.3.2. Co-inoculum Development: Formulate plant-bacterial co-inoculum and biostimulant for nitro-PAH degradation.

1.3.3. Technology Development: Establish bacterial and biostimulant-assisted phytoremediation for nitro-PAH removal in microcosms.