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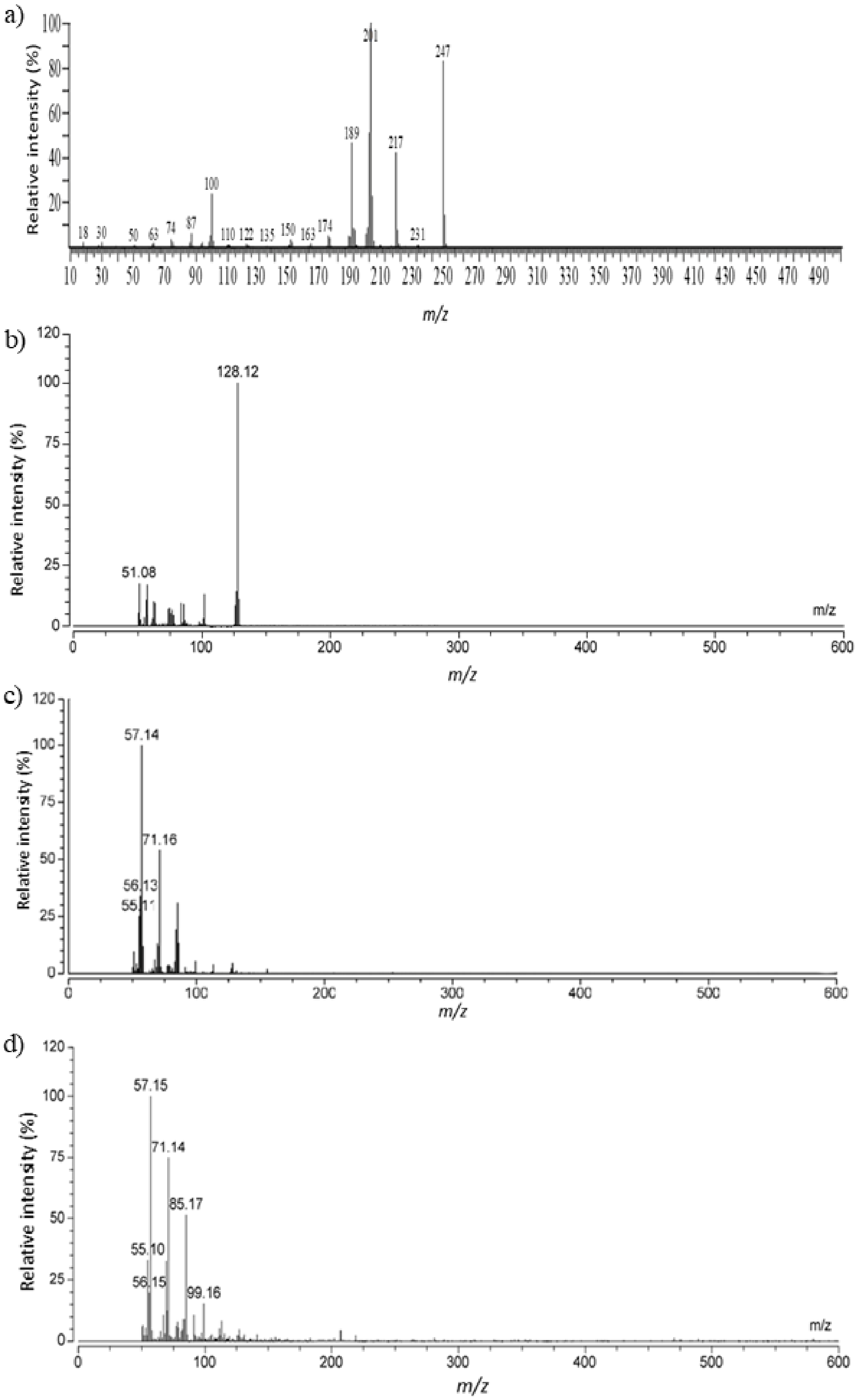
Appendix I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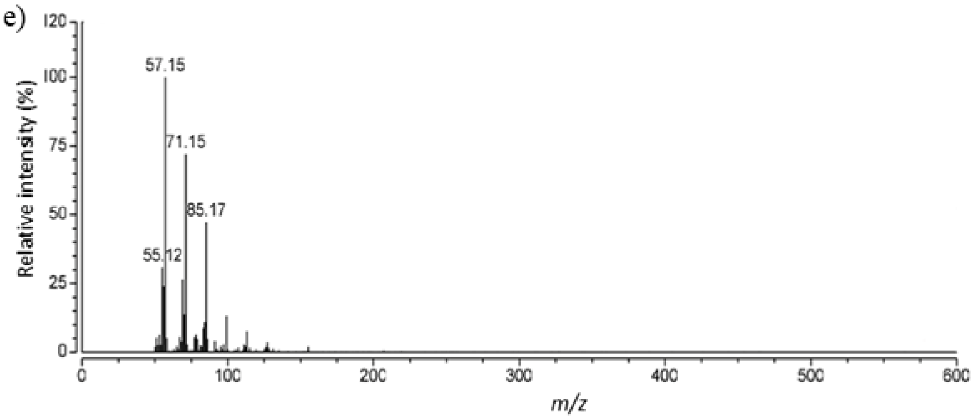

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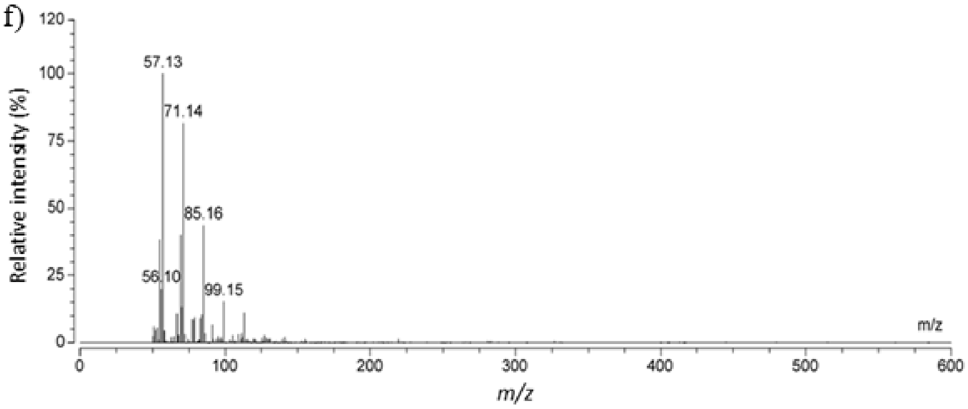

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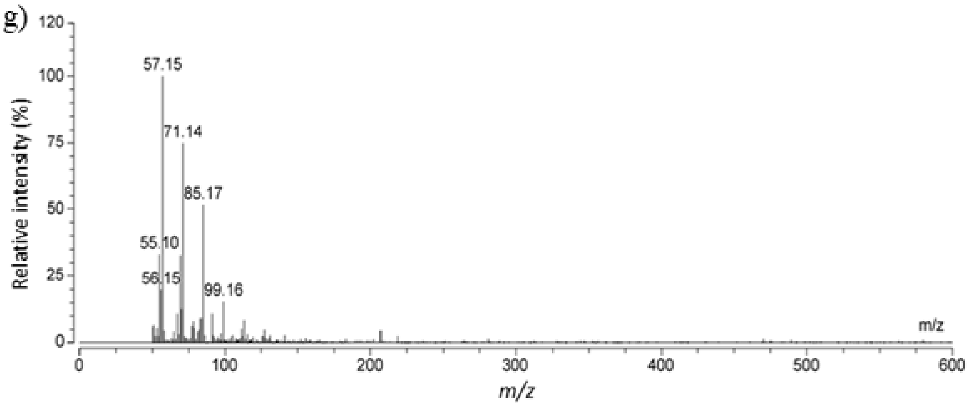

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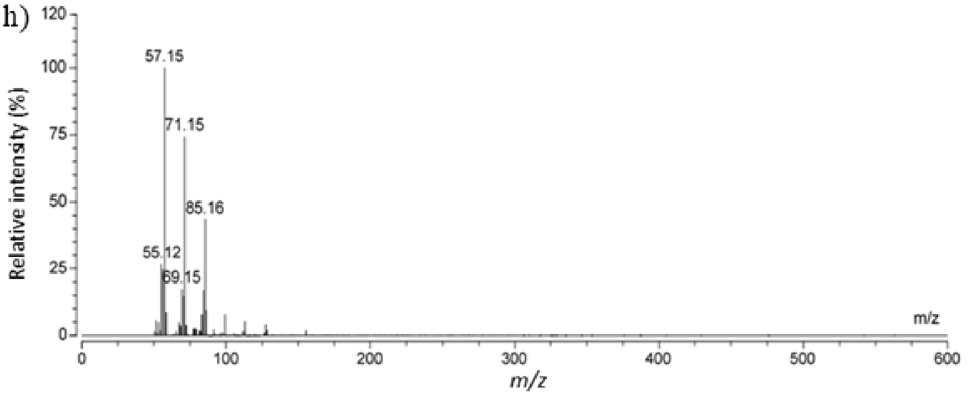

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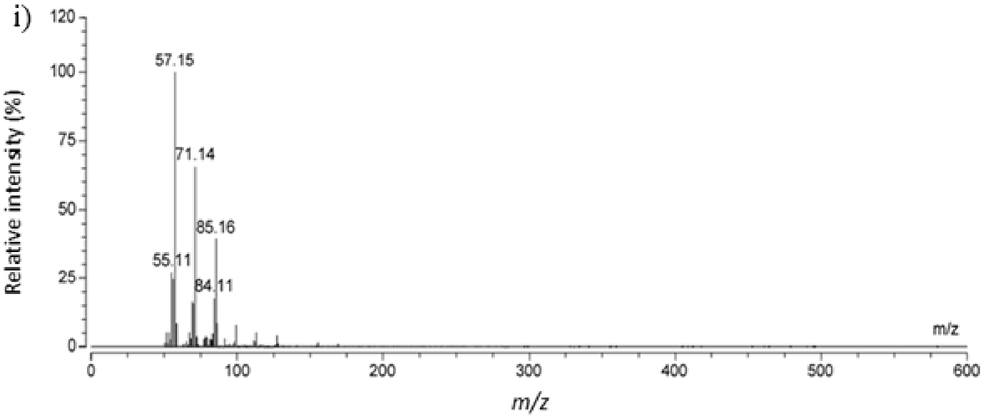

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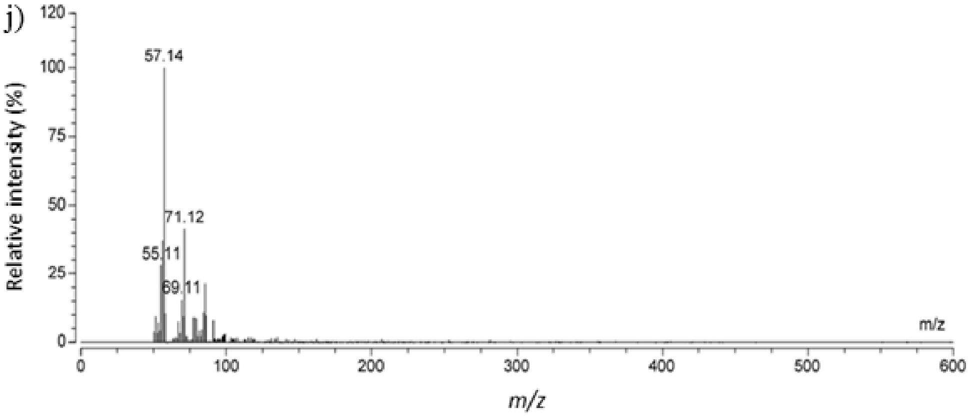

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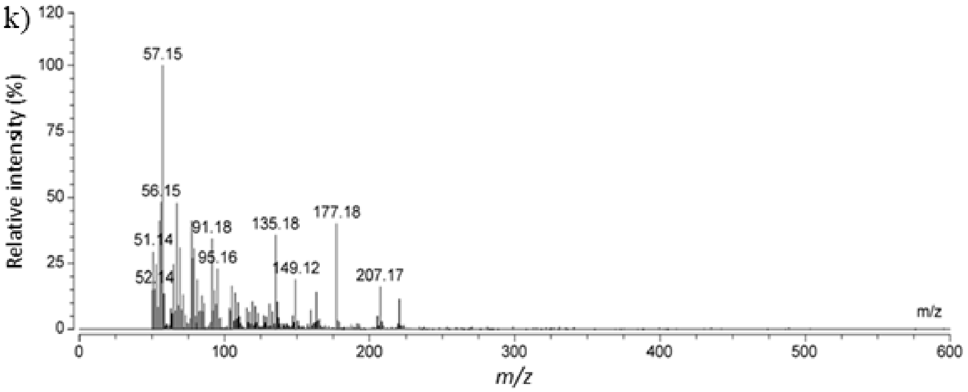

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200
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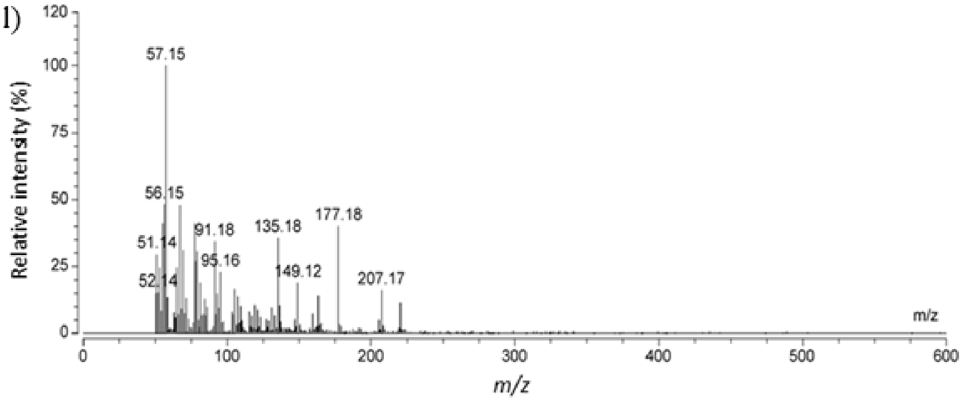
500
600
m/z

l)



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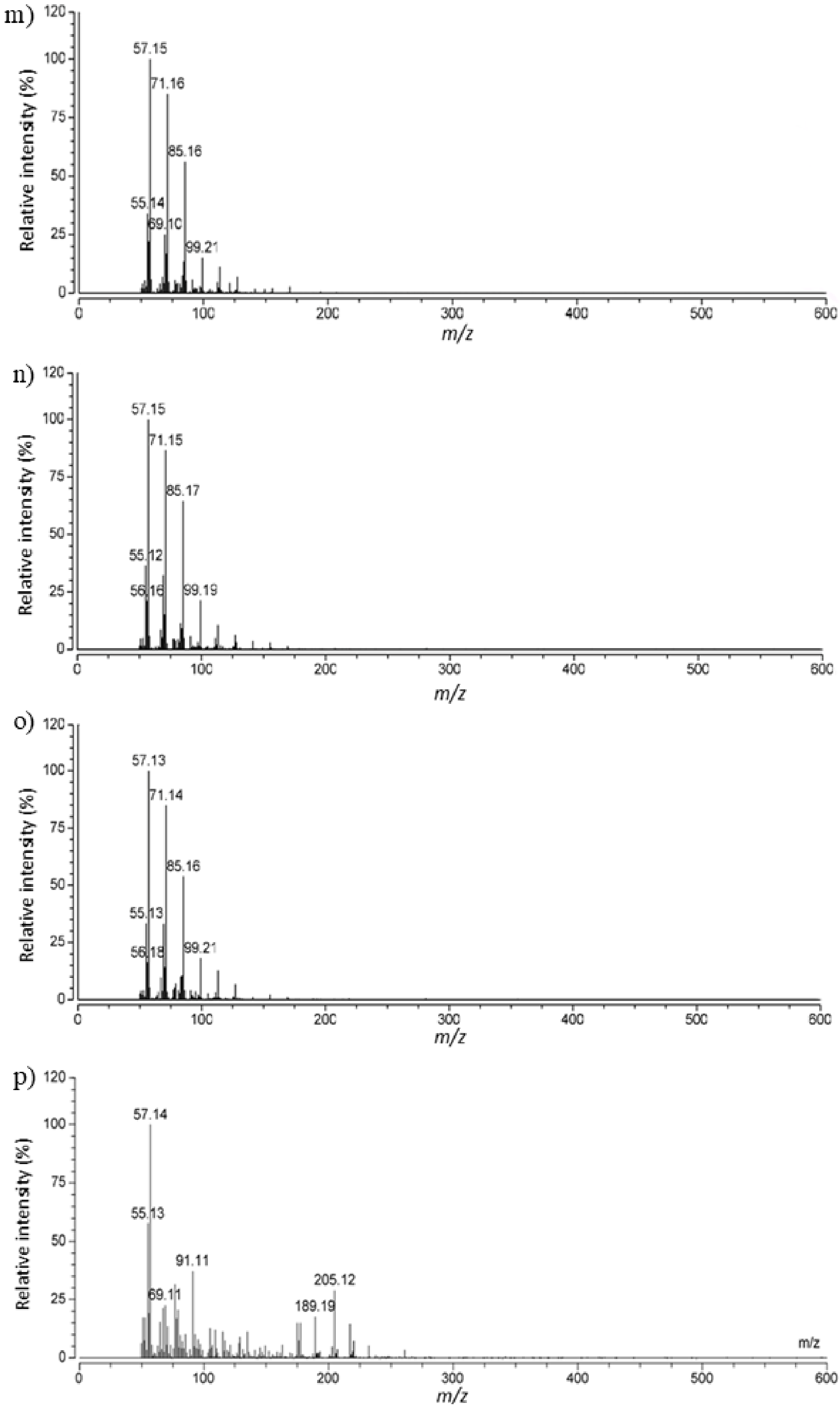
m)
Relative intensity (%)
m/z
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n)
Relative intensity (%)
m/z
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71.15
85.17
55.12
56.16
99.19
o)
Relative intensity (%)
m/z
57.13
71.14
85.16
55.13
56.18
99.21
p)
Relative intensity (%)
m/z
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55.13
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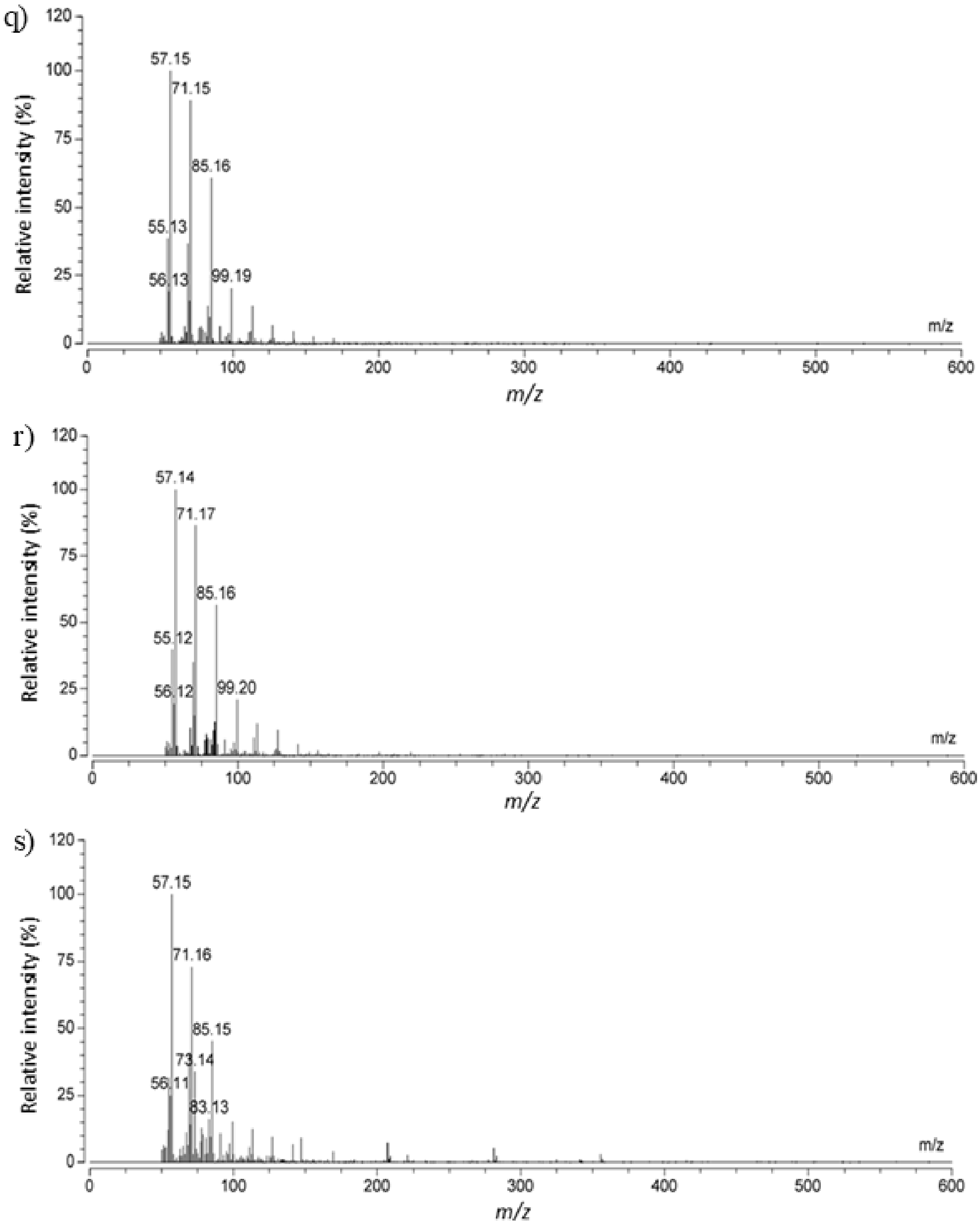
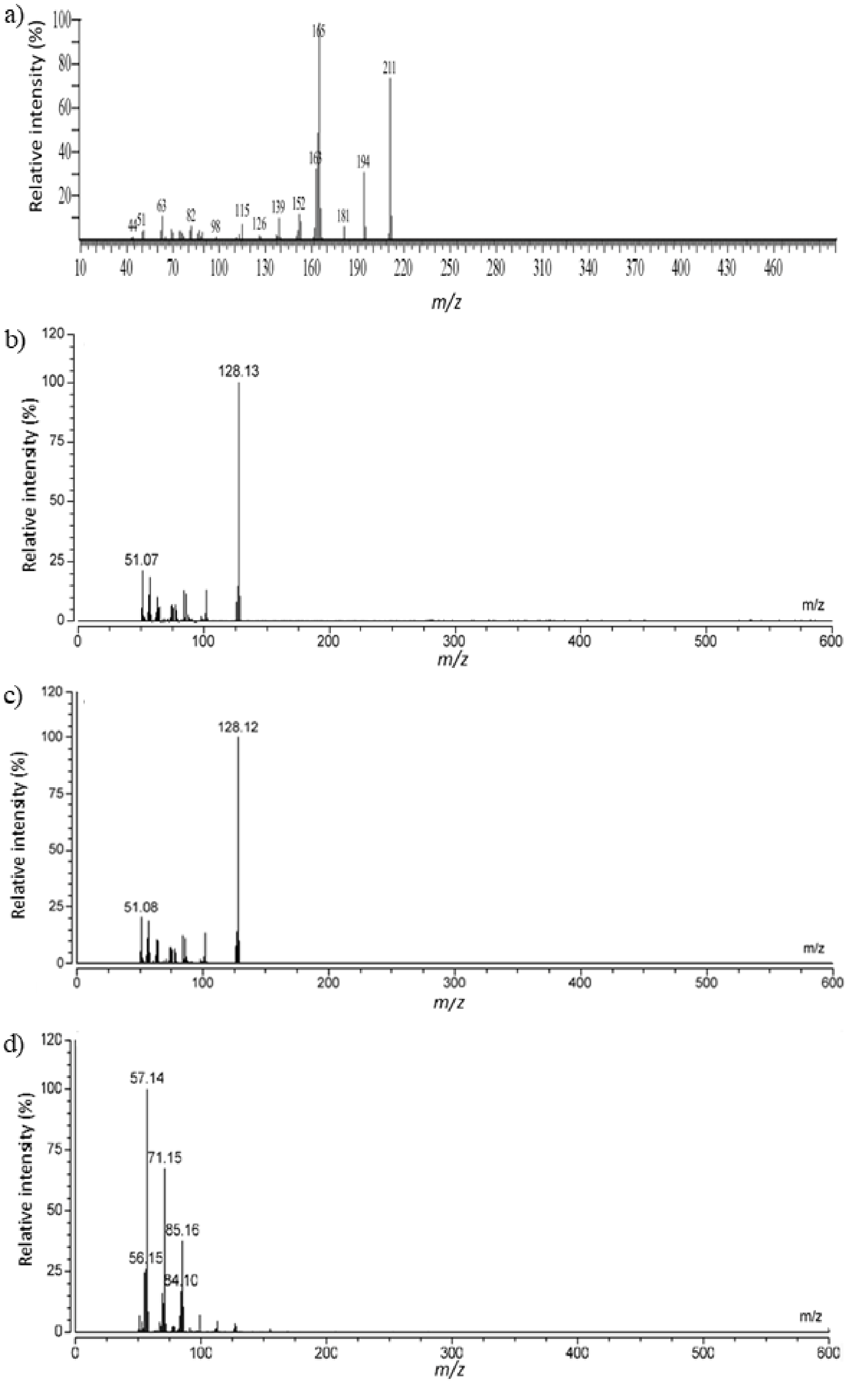


Fig. S1. Mass spectra depicting the analysis of 1-nitropyrene metabolites via GC-MS throughout the plant-bacterial co-inoculum and biostimulant treatment (a) baseline conditions (0 days), (b-g) following 30 days of incubation, and (h-s) following 60 days of incubation. The mass spectra demonstrate the progressive alterations in metabolite profiles throughout the degradation process.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63
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98
115
126
139
152
163
165
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194
211
10
40
70
100
130
160
190
220
250
280
310
340
370
400
430
460
m/z
b)
Relative intensity (%)
120
100
75
50
25
0
128.13
51.07
m/z
0
100
200
300
400
500
600
m/z
c)
Relative intensity (%)
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100
75
50
25
0
128.12
51.08
m/z
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100
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d)
Relative intensity (%)
120
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m/z
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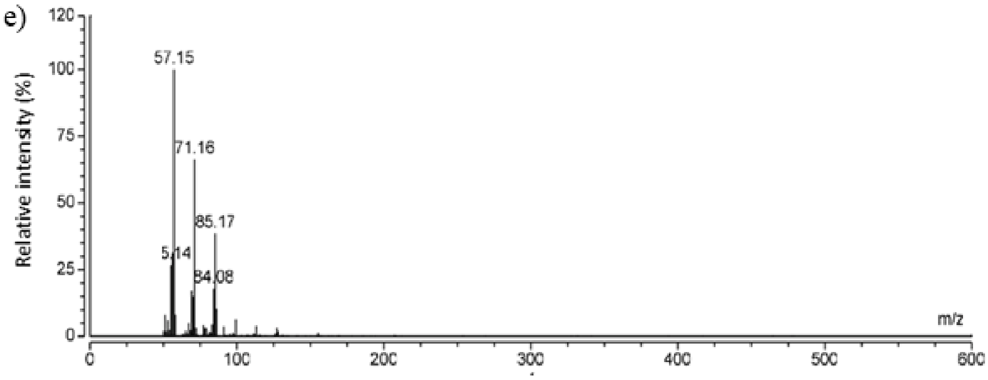
e)
Relative intensity (%)
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85.17
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84.08
m/z
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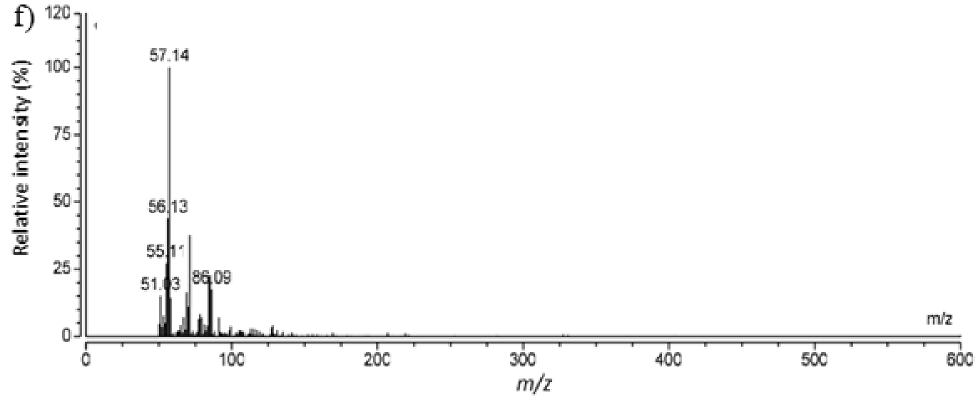
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Relative intensity (%)
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m/z
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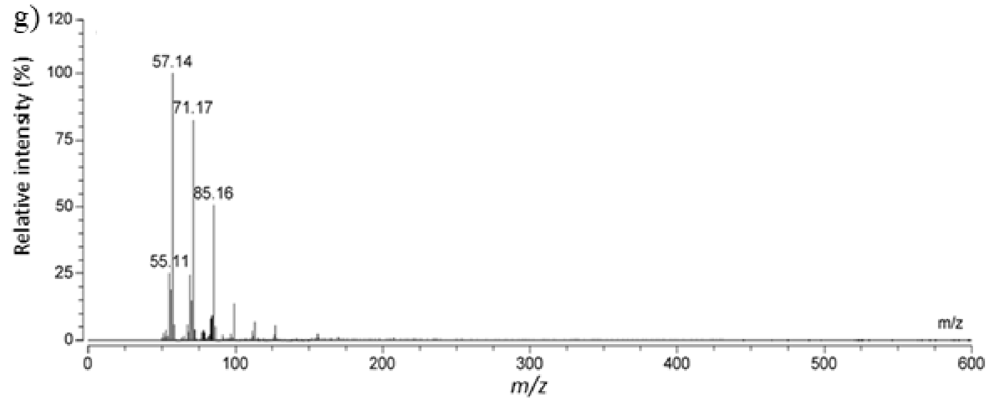
g)
Relative intensity (%)
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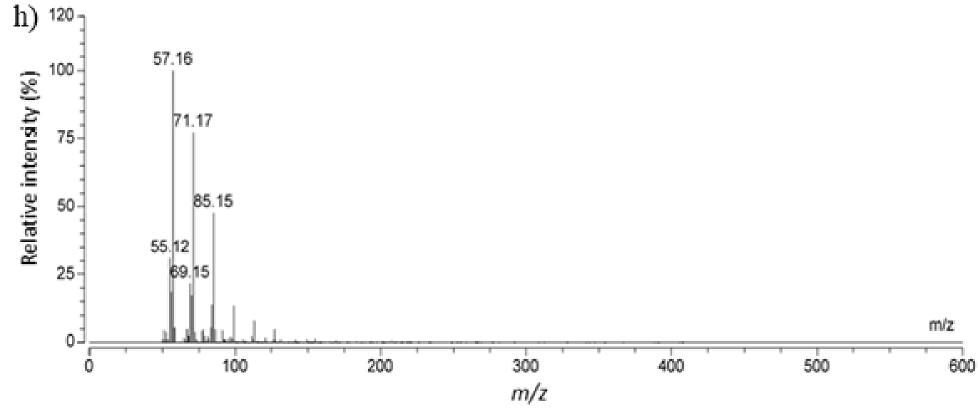
h)
Relative intensity (%)
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m/z
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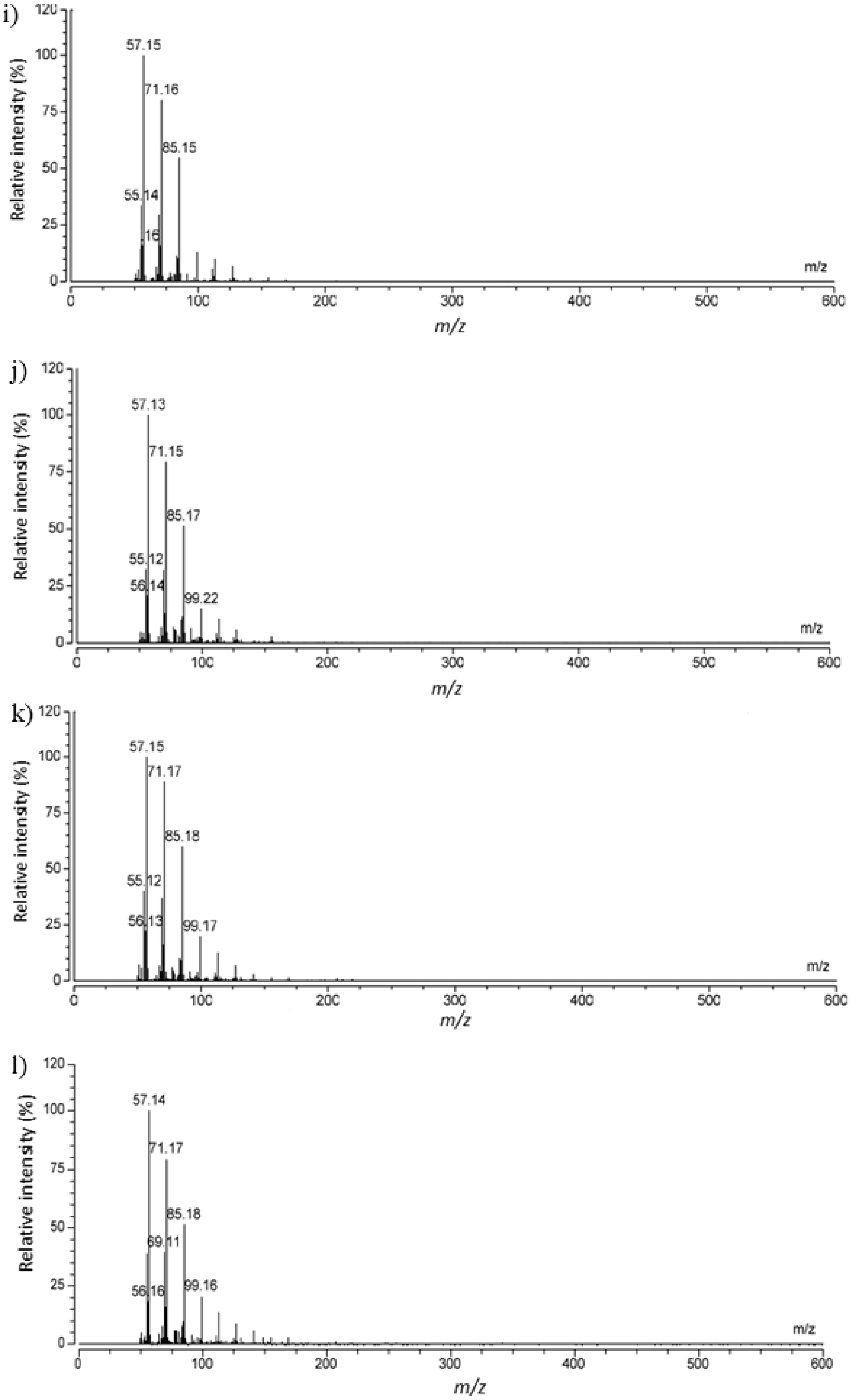

Fig. S2. The mass spectra of the metabolites via GC-MS throughout the plant-bacterial co-inoculum and biostimulant treatment: (a) baseline conditions (0 days), (b) following 30 days of incubation, and (c-l) following 60 days of incubation. The mass spectra demonstrate the progressive alterations in metabolite profiles throughout the degradation process.

Appendix II

PUBLICATIONS

1. Gogoi, B., Islam, N. F., & Sarma, H. (2025). Biodegradation of nitro-PAHs by multi-trait PGPR strains isolated directly from rhizosphere soil. The Microbe, 6, 100263. https://doi.org/10.1016/j.microb.2025.100263
2. Sarma, H., Gogoi, B., Guan, C.-Y., & Yu, C.-P. (2024). Nitro-PAHs: Occurrences, ecological consequences, and remediation strategies for environmental restoration. Chemosphere, 141795. https://doi.org/10.1016/j.chemosphere.2024.141795

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Biodegradation of nitro-PAHs by multi-trait PGPR strains isolated directly from rhizosphere soil

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ABSTRACT

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are hazardous, persistent organic pollutants widely distributed globally. They significantly threaten environmental health by degrading soil, water, and air quality. Prolonged exposure to nitro-PAHs increases risks for both humans and wildlife, leading to cancer, genetic mutations, endocrine disruption, neurodegenerative disorders, and oxidative stress. This study explored the degradation of nitro-PAHs using two plant growth-promoting rhizobacterial (PGPR) strains, *Bacillus cereus* BG034 and *Bacillus altitudinis* BG05, isolated from the rhizosphere of native plants (*Cyperus rotundus, Cyperus esculentus, Imperata cylindrica,* and *Axonopus compressus*). A co-inoculum (BGC01) formed from these bacterial strains of *Bacillus cereus* BG034 and *Bacillus altitudinis* BG05, demonstrated significant capabilities for degrading nitro-PAHs. After a 72-hour incubation period, BGC01 effectively removed 76.0 % of 1-nitropyrene and 87.2 % of 2-nitrofluorene. Individually, *Bacillus cereus* BG034 removed 47.8 % of 1-nitropyrene and 59.9 % of 2-nitrofluorene, while *Bacillus altitudinis* BG05 achieved the removal abilities of 49.0 % and 59.8 %. In addition to their degradation capacity, these bacteria exhibited traits that promote plant growth. These results emphasize the potential of these bacterial strains, particularly in co-inoculum form, as effective agents for nitro-PAH degradation. This study offers an environmentally friendly and cost-effective solution for environmental remediation and highlights the potential use of these bacteria as biofertilizers for sustainable agriculture.

1. Introduction

In recent decades, anthropogenic activities have led to the deterioration of the natural environment, posing many problems for ecosystems and human health. One of the most pressing environmental issues is the widespread discharge of nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) a class of PAHs produced by incomplete combustion of organic compounds or interactions with nitrogen oxides (NOx) in polluted settings. These compounds are far more harmful than their PAH precursor, with carcinogenic and mutagenic effects up to 12 and 10 times stronger, respectively (Lee et al., 2022; Yang et al., 2024). Vehicle emissions and oil spills are major factors, resulting from the processes of petroleum production, transportation, and refining (Sarma et al., 2024; Vasiljevic et al., 2021). According to Zhao et al. (2015), an explosion in an oil pipeline released several hazardous nitro-PAHs, including 1-nitropyrene, 2-nitrofluorene, 1,6-dinitropyrene, 1,8-dinitropyrene, 1,3-dinitropyrene, 6-nitrochrysene, 2-nitrofluoranthene, 6-nitrobenzene(a)pyrene, 3-nitrophenanthrene, 9-nitrophenanthrene, 9-nitroanthracene, 7-nitrobenzene(a)anthracene, and 1-nitronaphthalene (Zhao et al., 2015). Nitro-PAHs were found to occur widely in air, water, and soil. Their concentrations in the atmosphere vary significantly, with reported levels ranging from 0.078 to 11.7 ng m^{-3} in Birmingham, United Kingdom, and between 2 and 7.8 ng m^{-3} in Longyearbyen, Svalbard (Alam et al., 2015; Drotikova et al., 2020). In soils, nitro-PAHs are predominantly found along urban roadsides and within industrial zones, with concentration levels ranging from 29 to 158 ng g^{-1} in Xi'an, central China, to a notable high of 396–2530 ng g^{-1} in urban regions of Nepal (Wei et al., 2015; Yadav and Devi, 2021). Nitro-PAHs were also found to occur in water bodies, with a concentration of 19.7 ng L^{-1} in the Luchuan River, China, and higher concentrations also found in the Asano River, Japan (604 ng L^{-1}) (Chondo et al., 2013; Hung et al., 2012).

Once introduced into the ecosystem, nitro-PAHs become highly persistent and accumulate in the atmosphere, hydrosphere, and lithosphere over extended periods and pose serious risks to the environment and human health. Human exposure to nitro-PAHs occurs through inhalation, ingestion, and direct contact with the skin (Gao et al., 2022;

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Krzyszczak and Czech, 2021). In addition to their mutagenic and carcinogenic properties, they are also well-known for endocrine-disrupting, neurotoxic, and teratogenic properties (Zhang et al., 2021). Furthermore, these pollutants infiltrate water bodies, jeopardize aquatic organisms' health by disrupting reproduction and development, and bioaccumulate in food webs, ultimately affecting human consumers (Kong et al., 2023; Manzetti, 2012). Due to their resistance to degradation, nitro-PAHs remain in soil and water for extended periods. Moreover, nitro-PAHs significantly affect plant health through phytotoxicity, genotoxicity, oxidative stress, and metabolic disruption (Yang et al., 2024; Yun et al., 2019). Some plants, however, may respond to these obstacles through the production of detoxifying enzymes such as cytochrome P450, laccases, peroxidases, and glutathione S-transferase (Kathi, 2011; Molina and Segura, 2021). These enzymes aid in the breakdown of complicated nitro-PAHs by processes such as polymerization, hydroxylation, and epoxidation. They also immobilize contaminants by incorporating them into the soil's humic acids.

Therefore, various physical and chemical techniques have been devised to alleviate the environmental and health hazards associated with nitro-PAHs. Primarily physicochemical degradation involves photolysis and oxidative reactions with reactive species including hydroxyl radicals (•OH) and superoxide anions (O_2^-) facilitating the process. Nitro-PAHs' electron-deficient properties make them susceptible to attack by these radicals, resulting in the oxidation of nitro-PAHs (Fukuhara and Miyata, 1995). Chemical oxidants also play an important part in this process by promoting oxidation processes. Despite the promises of these methods, they face limitations. Consequently, there is a pressing need for sustainable and economically viable alternatives. Biological remediation, which employs plants and microorganisms to decompose intricate organic pollutants, presents a highly promising avenue. Among these microorganisms mediated remediation, plant growth-promoting rhizobacteria (PGPR) have garnered significant attention due to their dual functionality in pollutant degradation and enhancement of plant growth (Gogoi et al., 2024; Gupta et al., 2024). These bacteria, residing in the rhizosphere, possess specialized enzymes like monooxygenases, dioxygenases, nitroreductases, etc that effectively degrade contaminants by hydroxylation, nitro group reduction, and ring cleavage of nitro-PAHs, which ultimately convert nitro-PAHs into less toxic intermediates. These intermediates finally enter the Krebs cycle, where they are further broken down into carbon dioxide, water, and ATP, completing the mineralization process. This positions PGPR as an attractive, sustainable strategy for tackling both environmental pollution and agricultural productivity challenges. Their capacity to decompose hazardous substances while promoting plant growth provides a holistic approach to the management of nitro-PAHs and other enduring pollutants.

Research on the bacterial degradation of nitro-PAHs, particularly 1-nitropyrene and 2-nitrofluorene, has been limited. Therefore, the current study focused on isolating and identifying indigenous nitro-PAHs tolerant bacterial species from the rhizospheres of prevalent plant species in the Borhulla oil field, to effectively degrade these hazardous pollutants. Additionally, this study aims to test the collective efficacy of the isolated strains in enhancing degradation. This study also provides a roadmap for scaling up to field applications, emphasizing its practical significance. By emphasizing the dual capabilities of these strains, both in degrading nitro-PAH and enhancing plant growth, they are positioned as promising candidates for sustainable remediation.

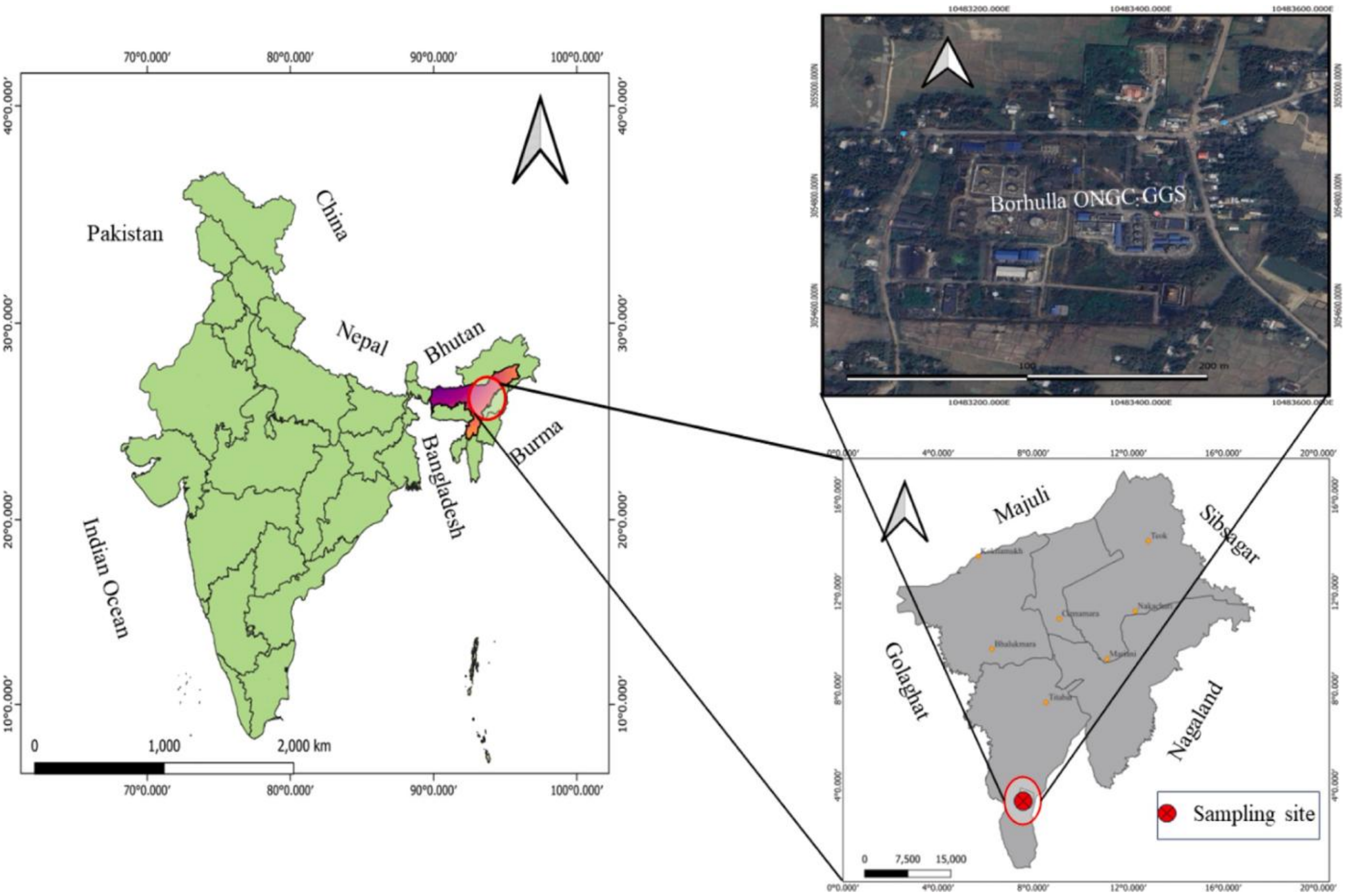

Fig. 1. Localization of sample site.

2. Materials and methods

2.1. Sampling site and chemicals

Samples were collected from the Borhulla oil fields in Upper Assam, situated at the coordinates 21° 45′ N and 94° 37′ E, to isolate PGPR (Fig. 1). Since 1976, the Oil and Natural Gas Corporation Limited (ONGC) has used this location for crude oil extraction and transportation, with occasional oil leaks reported. Nestled in northeastern India within the Indo-Burma biodiversity hotspot, the area features rich ecosystems. It receives 700–1500 mm of rain each year, with summers distinguished by high humidity and temperatures of up to 38°C and winters as low as 6°C (Deka et al., 2023). In this biodegradation experiment, 1-nitropyrene and 2-nitrofluorene were chosen as model nitro-PAHs because they are widespread occurrence and ecological significance (Wang et al., 2023; Zhou et al., 2022). These chemicals are typically detected in air, water, and soil; and recognized as hazardous pollutants. Their recurring emission during petroleum industry activities, along with their durability and possible toxicity, make them important subjects for this biodegradation study.

All of the media used in this study were purchased from Himedia. The Peptone, Beef Extract, and Yeast Extract (PBYS) medium was prepared with the following components (in g/L): 1 g yeast extract, 5 g peptone, 2.5 g beef extract, 0.01 g $FeCl_2$, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 50 g NaCl, 1 g K_2HPO_4, 0.5 g KH_2PO_4, 0.2 g $CaCl_2 \cdot 2\ H_2O$, 1 g NH_4NO_3, and 0.3 g KCl. The pH was adjusted to 7.0 ± 0.2 (Sarma et al., 2019). 1-nitropyrene and 2-nitrofluorene, both possessing 99 % purity, were procured from Sigma Aldrich, USA. Stock solutions of these chemicals were prepared with a concentration of 1000 mg/L in acetone and kept at 4°C for further use. All other chemicals and solvents utilized were of analytical grade.

2.2. Isolation and identification of indigenous nitro-PAHs tolerant PGPR strains from the rhizosphere of selected plant species

The rhizosphere soil was collected under sterile conditions from readily available plants in this sample site. The soil contained 0.9 % organic carbon, 265 kg/ha nitrogen, 22.4 kg/ha phosphorus, 97.08 kg/ha potassium, and had an electrical conductivity of 0.10 dS/m with a pH of 5.09. The collected soil samples were promptly transported to the laboratory in an ice box to maintain their quality and subsequently air-dried in a shaded area. The dried soil was sifted through a 2 mm sieve to remove debris and kept at 4°C for future experiments.

PGPR isolation was accomplished by utilizing the serial dilution plate method. The soil sample was diluted (10^{-8}) and plated onto nutrient agar plates supplemented with nitro-PAHs, which acted as the carbon source. The plates were prepared in triplicate, with nitro-PAHs, specifically 1-nitropyrene and 2-nitrofluorene, and applied individually to the media as a 2 % stock solution after soil inoculation. After incubation at 37°C for 48 hours, the number of bacterial colony-forming units per gram (CFU/g) of soil for the selected plant species was determined. The two colonies that formed the largest clear zones, indicating efficient nitro-PAH degradation, were isolated for further studies (Sarma et al., 2019).

Two approaches were used to thoroughly identify isolated PGPR strains. Gram staining was used to determine the morphological properties of the PGPR. DNA was extracted from each isolated bacterial strain for molecular identification using Qiagen's DNeasy PowerSoil Pro Kits. The 16S rDNA region, a widely recognized marker for bacterial identification, was amplified via polymerase chain reaction (PCR) with Taq DNA polymerase and universal bacterial primers 27 F and 1492 R, which are designed to target conserved areas of the bacterial 16S rDNA gene. The amplified DNA fragments were ligated into a vector and sequenced on an ABI 3730xl Genetic Analyzer with vector primers and the BDT v3.1 Cycle Sequencing Kit. The retrieved sequences underwent BLAST analysis and were compared with sequences available in the GenBank database through the NCBI BLAST server (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rDNA sequences of these two PGPR strains were later submitted to GenBank under accession number PQ394621 for *Bacillus cereus* BG034 and PQ390296 for *Bacillus altitudinis* BG05.

Phylogenetic trees and evolutionary relationships derived from the 16S rDNA sequences were built employing neighbor-joining and maximum likelihood approaches in MEGA11 (Molecular Evolutionary Genetics Analysis). The resulting phylogenetic tree was subsequently exported to iTOL (https://itol.embl.de/) for visualization.

2.3. In vitro screening of plant growth-promoting traits

Various qualitative assays were conducted to evaluate the plant growth-promoting characteristics of the isolated strains. Phosphate solubilization was assessed by using tricalcium phosphate as an insoluble phosphate source on Pikovskaya's agar medium augmented with bromophenol blue (0.024 mg/mL), and the emergence of a yellow halo surrounding bacterial colonies after 72 hours of incubation at 37°C indicated positive phosphate solubilization (Pande et al., 2017). The production of indole acetic acid (IAA) was determined using Mu'minah et al. (2015) methodology, which involved incubating bacteria cultures in L-tryptophan-supplemented nutrient broth (NB) medium at 28°C at 72 hours and then reacting with orthophosphoric acid and Salkowski's reagent (50 mL, 35 % H_2SO_4, 1 mL of 0.5 M solution of $FeCl_3$) to produce a pink coloration, confirming IAA synthesis (Mu'minah et al., 2015). Hydrogen cyanide (HCN) production was assessed using glycine-supplemented nutrient agar slants, with filter paper saturated with 2 % sodium carbonate and 0.5 % picric acid; after incubation of bacteria inoculated slants at 30°C for 96 hours a color change in the filter paper from orange to red indicated positive HCN production (Sani et al., 2024). Ammonia production was assessed by incubating bacterial cultures in peptone water at 37°C for 72 hours, then adding Nessler's reagent, which resulted in a change from brown to yellow, indicating confirmed ammonia synthesis (Cappuccino and Sherman, 1992). The Triple Sugar Iron (TSI) test assessed the fermentability of bacterial strains using three different sugars: glucose, sucrose, and lactose. The isolated bacteria strains were inoculated into the TSI agar tubes and incubated at 30°C for 48 hours. A yellow color in the medium indicated sucrose and lactose fermentation, but a yellow butt showed glucose fermentation. A broken agar surface also stated the production of CO_2 gas, while the formation of a black precipitate represented the synthesis of ferrous ammonium sulfate (Lehman, 2005). The oxidase test, performed using the Filter Paper Spot Method, involves treating bacterial colonies with 1 % Kova´cs oxidase reagent (1 % tetra-methyl-p-phenylenediamine dihydrochloride, in water) until a dark purple color appeared within 5–10 seconds, indicating a positive result (Shields and Cathcart, 2013). The catalase test involved adding H_2O_2 to an 18-hour-old isolated bacterial culture, resulting in the instantaneous appearance of bubbles, indicating catalase activity (Reiner, 2013). Gelatinase activity was assessed by incubating bacterial cultures in nutrient agar tubes containing gelatin at 28°C for 48 hours. The liquefaction of the tubes after cooling revealed positive gelatinase activity (dela Cruz and Torres, 2012).

2.4. Greenhouse evaluation for PGPR

An *in vivo* greenhouse experiment using a randomized block design to evaluate the plant growth-promoting properties of the isolated PGPR strains. The isolated PGPR strains were initially grown in NB medium at 37°C for 12 hours at 200 rpm. A co-inoculum (BGC01) was then prepared using bacterial strains BG034 and BG05. Prior to preparing the co-inoculum, bacterial strains were tested for their compatibility following the methodology outlined by Irabor and Mmbaga (2017). In brief, each bacterial strains were cultured individually in a NB medium overnight at 28°C and 200 rpm. Following that, 100 μL of the test bacterial strains

BG034 and BG05 were dispersed individually on the surface of nutrient agar plates with a population of around 10^8-10^9 colony-forming units per mL (CFU/mL). Filter paper discs with a diameter of 1 cm (Whatman number 1) were put on the spread plate. Each disc was inoculated with an overnight NB culture of the strains being evaluated for compatibility. The plates were subsequently incubated at 28°C for 4 days and monitored their growth pattern at 24-hour intervals (Irabor and Mmbaga, 2017). Equal volumes of each bacterial suspension were combined to prepare the co-inoculum for further experimentation. 10 mL of each bacterial culture and BGC01 were again inoculated in NB medium and incubated at 37°C until the exponential growth phase was reached. The cells of each culture were separated through centrifugation at 1000 ×g for 5 minutes and rinsed three times with sterile water. The absorbance of the cell suspensions was adjusted to a range of 0.3–0.4 at 600 nm using a UV-Vis spectrophotometer. This ensured uniform cell density for subsequent experiments. Concurrently, the most prevalent plant species *Cyperus rotundus* L., *Cyperus esculentus* L., *Imperata cylindrica* (L.) Beauv., and *Axonopus compressus* (Sw.) P. Beauv. which were screened out from the study site, previously maintained in the institute experimental garden, were transplanted into pots containing sterilized soil. The prepared culture, at a final density of 4.7×10^{13} CFU/mL, was applied to the soil around the seedlings at 100 mL per plant. Control plants received an identical sterile NB medium free of PGPR strains. The plants were nurtured within a greenhouse under regulated conditions, including a 25–28°C temperature range, a photoperiod of 16 hours of light and 8 hours of darkness, and systematic watering to maintain soil moisture. The plant growth parameters, such as shoot length and root length, were measured at 7, 14, and 21-day intervals. The effectiveness of the PGPR strains and their co-inoculant was assessed by comparing the growth metrics of treated and control plants. To minimize standard error, measurements were taken from three replicates for each treatment group.

2.5. *Assessment of nitro-PAHs degrading potential of isolated PGPR strains in shaker flask*

The isolated PGPR strains (BG034 and BG05) were initially inoculated into NB medium and incubated at 200 rpm at 37°C for 12 hours. Following the incubation period, the bacterial cultures were centrifuged at 8000 ×g for 10 minutes at 4°C, and the resultant pellets were washed three times with sterile distilled water to remove any residual media. The pellets were then resuspended in a minimal salt medium. McFarland's turbidity standard was calibrated. The bacterial cultures were adjusted to a concentration of 50,000 cells per mL. For the determination of *in vitro* cell growth, bacterial suspensions (10 mL of individual strains and a mixture of 5 mL BG034 and 5 mL BG05 as a co-inoculum, BGC01), were inoculated to a 1-nitropyrene-PBYS medium and 2-nitrofluorene-PBYS medium prepared at varying nitro-PAH concentrations (12.5 mg/L, 25 mg/L, 37.5 mg/L, and 50 mg/L). Before inoculation, the medium was left uncovered in a laminar flow cabinet for 12 hours to let the acetone evaporate. An abiotic control was maintained throughout the experiment, consisting of the same nitro-PAHs-PBYS medium but no bacterial inoculations. Following inoculation, the flask was kept at 37°C and shaken at 200 rpm. At 7-day incubation periods, the bacterial biomass in the flasks was measured turbidimetrically at 600 nm using a UV-Vis spectrophotometer. The bacterial biomass (grams of dry cell weight per liter) was calculated by using the formula: Biomass (g/L) = 0.5413 × absorbance (Moscoso et al., 2012). The complete experiment was carried out three times to ensure reliability and accuracy. Based on the results of the growth experiment, the threshold concentration for the growth of bacteria was established, leading to the preparation of 1-nitropyrene-PBYS medium and 2-nitrofluorene-PBYS medium at a final concentration of 25 mg/L for subsequent degradation experiment. Adhering to the aforementioned inoculation protocol, bacterial strains, and the co-inoculum were introduced into the medium. An abiotic control was maintained throughout the experiment, consisting of the same nitro-PAHs-PBYS medium but no bacterial inoculations. Following inoculation, the flask was kept at 37°C and shaken at 200 rpm. At 0, 24, 48, and 72-hour intervals, 15 mL samples were collected from the individual shaker flask. These samples underwent centrifugation at 8000 ×g for 15 minutes at 4°C. The resulting supernatant was collected and thoroughly filtered to remove any remaining bacteria. The resulting filtrate underwent liquid-liquid extraction, mixing it with an equal volume of acetone and hexane in a 20:80 (v/v) ratio. Anhydrous sodium sulfate was added to the mixture to remove any leftover water from the organic phase, which was then filtered again. The resultant samples were analyzed using high-performance liquid chromatography (HPLC) with a 4288 C 1220 Infinity II Gradient L.C. System V.L. equipped with column (C18 (2), 4.6 × 250 mm,5 μm) to quantify residual nitro-PAHs. Before analysis, a mobile phase of acetonitrile and water in an 85:15 ratio was prepared and degassed for 15 minutes with an ultrasonic bath sonicator. Simultaneously, 1 mg of 1-nitropyrene and 2-nitrofluorene was dissolved in 1 mL of acetonitrile to prepare standard stock solution. Working standard solutions were prepared by diluting stock solutions with acetonitrile to achieve concentrations of 5, 10, and 20 μg/mL, and their standard chromatograms are presented in Figure S9. To get the concentrations necessary for the standard calibration curve (Figures S7 and S8), serial dilutions were used. Additionally, 100 μL aliquot of each sample (2 mL total volume) was diluted in microcentrifuge tubes with 900 μL of acetonitrile. After sonicating for 10 minutes, the samples were filtered through 0.2 μm, 13 mm nylon membrane filters before being introduced into the HPLC for analysis. The nitro-PAHs concentration (mg/L) was determined from the HPLC spectra and their biodegradation percentage was determined using the following formula:

$$\text{Degradation\%} = \frac{\textit{Initial concentration} - \textit{Final concentration}}{\textit{Initial concentation}} \times 100\%$$

Simultaneously, samples were collected at 0, 15, and 30-day intervals from BGC01 treated nitro-PAHs PBYS medium to assess potential metabolites produced by the selected bacterial co-inoculum. Samples were prepared using a liquid-liquid extraction method with acetone and hexane in a 20:80 (v/v) ratio. The extracted samples were further concentrated and GC-MS analysis was carried out using an Agilent 7890B Gas Chromatography system and an Agilent 5977 A Mass Selective Detector equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL/min, and sample injections (1 μL) were done in split mode with a split ratio of 10:1. The temperature program for the oven commenced at 50°C with a stabilization period of 2 minutes, subsequently rising at a rate of 10°C per minute until reaching 150°C, where it was held for an additional 2 minutes. The temperature was then elevated at a rate of 5°C per minute to 250°C, followed by a 5 minutes stabilization, and ultimately, it was increased at 10°C per minute to 280°C, maintaining a hold for 5 minutes. The injector and transfer line temperatures were established at 250°C and 100°C, respectively. In mass spectrometry, the temperature of the ion source was set at 230°C, while the temperature of the quadrupole was kept at 150°C. The mass range was set from 35 to 500 *m/z*, with a solvent delay of 3 minutes and a scanning rate of 1.562 scans per second. Compounds were identified by comparing retention durations and mass spectra to known compounds in the NIST 20.1 (2020) database, using the NIST library search (C:\Database\NIST20.1).

2.6. *Statistical analysis*

The dataset was thoroughly statistically evaluated to determine each treatment's mean and standard deviation (S.D.). The statistical analysis was performed using PAST 4.3 to investigate significant differences in treatment effects over time. For normally distributed data, one-way ANOVA was performed, while the Kruskal-Wallis test was used for skewed data to determine significant differences ($p < 0.05$) between the treatment cohorts.

3. Results and discussion

3.1. Isolation and characterization of nitro-PAHs tolerance PGPR strains

In this study, four prominent plant species belong to the Poaceae family, i.e., *Cyperus rotundus* L., *Cyperus esculentus* L., *Imperata cylindrica* (L.) Beauv., and *Axonopus compressus* (Sw.) P. Beauv. (Figure S1) were screened out due to their widespread availability, ease of cultivation, and notable presence in this polluted environment. Furthermore, the hyperaccumulating properties of these plants were confirmed using the PHYTOREM database, but their specific capacity to remediate nitro-PAHs had not been determined. The survival and occurrence of these species in this contaminated region demonstrate their flexibility and significant interactions with some nitro-PAHs-tolerant PGPR, which most likely contribute to their resilience in polluted soils. Significant variations in PGPR colonization, assessed as CFU/g, were found among the species when their rhizosphere soil was inoculated in 1-nitropyrene nutrient agar and 2-nitrofluorene nutrient agar. *Axonopus compressus* had the maximum bacterial colonies, with CFU/g values of 117.30 on 1-nitropyrene and 108.35 on 2-nitrofluorene agar. In contrast, *Imperata cylindrica* and *Cyperus rotundus* had lower CFU/g values, with *Imperata cylindrica* at 57.33 and *Cyperus rotundus* at 45.66 on the respective medium. The results highlighted the richness of PGPR colonies among the selected species. It is hypothesized that the selected grass species have significant potential for facilitating nitro-PAH's microbial breakdown in polluted soils. This establishes them as viable candidates for future bioremediation research.

Bacteria were isolated from the rhizosphere soils of the selected plant species using the serial dilution plate technique. The gathered rhizosphere soil samples were mixed, and a dilution of 10^{-8} was dispersed over nitro-PAHs-nutrient agar, leading to a diverse population of bacteria. Two pure colonies were successfully isolated from this population based on their ability to form clear zones on nitro-PAHs-PBYS agar plates, where 1-nitropyrene and 2-nitrofluorene acted as the sole carbon sources in the absence of supplemental nutrients, with the clear zones indicating the bacteria's ability to breakdown these compounds. The BLAST analysis revealed that one of the isolates shared 99.66 % similarity with the 16S rDNA gene sequence of *Bacillus cereus* reported in GenBank, while another shared 99.15 % identity with *Bacillus altitudinis*. The phylogenetic tree is depicted in Figure S2, showcasing the evolutionary lineage of these strains.

3.2. In vitro plant growth-promoting traits of the isolates

The *in vitro* results of this experiment are presented in Figure S3 which demonstrated the presence of PGPR features in the isolated rhizosphere bacteria i.e., *Bacillus cereus* BG034 and *Bacillus altitudinis* BG05. Both strains demonstrated to solubilizing phosphate, an essential nutrient for plants. The formation of yellow (i.e. phosphate solubilization) zones around the colonies indicated that strains of *Bacillus cereus* and *Bacillus altitudinis* can solubilize insoluble inorganic phosphate (Bharti et al., 2024; Ganesan et al., 2024). Though phosphorus is abundant in soil, it frequently exists in forms inaccessible to plants, particularly in polluted areas where phosphate fixation and precipitation reduce its solubility. PGPRs improve phosphate availability by mineralizing organic phosphate and solubilizing inorganic phosphate complexes (Bakki et al., 2024; Gurav et al., 2024). Both isolated strains showed favorable outcomes in the IAA assay, demonstrating their capacity to produce this crucial phytohormone. IAA is well regarded for its ability to stimulate root growth, cellular proliferation, and overall plant development (Jin et al., 2023). The ability of these bacterial strains to produce IAA indicates that they have the potential to directly promote plant development via improving root structure. HCN formation, another notable characteristic that promotes plant growth and development, was detected in both isolates. HCN synthesis is usually associated with biological plant pathogen management because it can inhibit the development of harmful bacteria in the rhizosphere (Sehrawat et al., 2022). This trait suggests that these strains may help reduce plant diseases and significantly increase the percentage of germination, nutrient uptake capacity, and biomass. Furthermore, both bacterial strains produced ammonia, highlighting their possible role in nitrogen cycling in the soil. Ammonia production is critical for supplying nitrogen to host plants, hence increasing their overall growth and development (Dietz et al., 2024). Ammonia rapidly reacts with soil water to form ammonium ions, providing an immediately accessible nitrogen source for plant uptake (Follett, 2001). This enhanced nitrogen availability promotes root and shoot elongation, resulting in to promotion of root and shoot elongation and their biomass (Bhattacharyya et al., 2020). Moreover, the TSI test yielded good results for both strains, as seen by the yellow color of the medium, indicating that they can ferment sucrose. This fermentative aptitude reflects the strains' metabolic versatility, allowing them to thrive in various soil conditions and contribute to the carbon cycle through organic matter breakdown. Furthermore, both isolates tested positive for oxidase and catalase, showing well-oxidative metabolic pathways (Chavan et al., 2022). These properties suggest that the isolates are well-suited to live in oxygen-rich settings, boosting their potential as plant growth promoters (Saeed et al., 2023). The gelatinase test revealed good activity in both strains, showing their ability to hydrolyze gelatin and potentially break down other protein-based compounds in the soil. This proteolytic activity may significantly impact nutrient cycling, increasing the availability of amino acids and other organic molecules for plant absorption (Costa et al., 2023).

3.3. Greenhouse evaluation for PGPR

The isolated bacterial strains and their co-inoculum ability to stimulate plant development were examined using *in vitro* screening and greenhouse trials. These experiments aimed to assess their impact on crucial growth variables such as shoot length and root length. The greenhouse results supported the favorable effects *in vitro*, demonstrating that these strains can promote plant growth. During the 21-day treatment intervals, significant statistical differences ($p < 0.05$) were observed between the co-inoculum and the individual strains treatments (From 7 days to 21 days, as detailed in Table 1) when they were administered to four selected plant species (*Cyperus rotundus, Cyperus esculentus, Imperata cylindrica,* and *Axonopus compressus*) compared to the control group (Fig. 2). Nonetheless, no significant differences were found between the strains' (BG034 and BG05) treatments. The findings of this study unequivocally corroborate previous research that has highlighted the plant growth-promoting properties of *Bacillus altitudinis* and *Bacillus cereus*. Yue et al. (2022) revealed that *Bacillus altitudinis* significantly increased both root and shoot lengths in wheat plants, demonstrating its ability to positively alter important growth metrics (Yue et al., 2022). Similarly, Kaushal and Pati (2024) reported significant increases in the root and shoot lengths of rice seedlings after inoculation with *Bacillus altitudinis*, supporting its plant growth-promoting properties effect across a wide range of crops (Kaushal and Pati, 2024). The plant growth-promoting properties attributes of *Bacillus cereus* have also been extensively documented in previous studies (Naseem et al., 2024; Sahile et al., 2021). Notably, Zhao et al. (2024) revealed the effectiveness of *Bacillus cereus* isolated from cyanobacterial crusts by significantly enhancing growth parameters in maize seedlings, notably shoot and root lengths (Zhao et al., 2024). The findings of the current study, when considered alongside these earlier observations, affirm the two isolated strains' potential as efficient PGPR. Moreover, combining both strains, i.e., co-inoculum (BGC01), showed the most spectacular outcomes. Plants treated with the co-inoculum showed significantly higher shoot and root length increases than those treated with individual strains. The combined inoculum's improved

Table 1

Results comparing differences in shoot and root lengths among plant species over the 7–21 days experimental period.

Plant's growth parameter	Plant species	Distribution pattern	Degrees of freedom (df)	Chi-square (χ2) / F-value	Significance value (p)
Shoot length	*Cyperus rotundus*	Skewed distribution	3, 32	9.35	0.025
	Cyperus esculentus	Skewed distribution	3, 32	8	0.046
	Axonopus compressus	Skewed distribution	3, 32	15.68	0.001
	Imperata cylindrica	Normally distributed	3, 32	4.14	0.014
Root length	*Cyperus rotundus*	Normally distributed	3,32	3.08	0.041
	Cyperus esculentus	Skewed distribution	3, 32	8.67	0.033
	Axonopus compressus	Normally distributed	3, 32	5.68	0.003
	Imperata cylindrica	Normally distributed	3, 32	5.24	0.005

growth was most likely due to the two strains' synergistic interaction, which was facilitated by complementary metabolic functions such as nutrient solubilization, growth-promoting hormone production, gelatine hydrolyzation, and soil pathogen inhibition via mechanisms such as hydrogen cyanide production. The previous report revealed that using a co-inoculum of PGPR bacterial strains not only enhances plant development but also uses the benefits of numerous microorganisms, resulting in a more robust support system for plants (Boonmahome et al., 2023; Devi et al., 2024). The bacterial co-inoculum's ability to promote plant development, particularly in polluted soils. These findings suggest that it may be used in agricultural techniques to increase crop yields while decreasing reliance on chemical fertilizers.

3.4. Degradation dynamics of nitro-PAHs by multi-trait PGPR strains

This study investigates the ability of multi-trait PGPR strains to degrade two model persistent nitro-PAHs, 1-nitropyrene, and 2-nitrofluorene, which are commonly found in contaminated environments. The strains, which were isolated from contaminated rhizosphere soils, were chosen for their resilience and potential to degrade these nitro-PAHs. The strains were grown in nitro-PAHs-PBYS medium, where nitro-PAHs serve as the sole carbon source. The concentrations of 1-nitropyrene and 2-nitrofluorene in the PBYS medium were standardized to a predetermined value at the onset of the experiment. The standardized concentrations (25 mg/L) provided a benchmark against which the effectiveness of the PGPR strains could be assessed. Nitro-PAH concentrations (mg/L) were monitored throughout time and Fig. 3 displays degradation patterns for both individual strains and the co-inoculum (BGC01). The degradation kinetics of 1-nitropyrene and 2-nitrofluorene demonstrated distinct patterns in our study underscoring the variations in their kinetics pathways. A second-order kinetic decay model better suited for 1-nitropyrene degradation, with R^2 values: 0.986 for BG034, 0.986 for BG05, and 0.997 for the co-inoculum BGC01. 2-nitrofluorene degradation followed a first-order kinetic decay model, with R^2 values of 0.986 for BG034, 0.994 for BG05, and 0.997 for BGC01, showing different microbial degradation dynamics between the two chemicals. In the degradation kinetic of 1-nitropyrene, the co-inoculum BGC01 had the highest rate constant ($k = 0.0034$ L mol^{-1} h^{-1}) and the shortest half-life ($T_{1/2}$ = 11.76 hours) compared to the individual strains BG034 and BG05, which both presented lower rate constants ($k = 0.0011$ L mol^{-1} h^{-1}) and longer half-lives ($T_{1/2}$ = 36.36 hours). The higher R^2 values (0.997 for BGC01) suggest that the second-order kinetic model is more effective and have higher degradation efficiency compared to the individual strain's treatment. Similarly, in the case of 2-nitrofluorene, the co-inoculum BGC01 also had the higher degradation efficiency with a rate constant of 0.0284 L mol^{-1} h^{-1} and a half-life of 33.90 hours. In contrast, individual strains BG034 and BG05 had lower rate constants (0.0122 L mol^{-1} h^{-1} and 0.0124 L mol^{-1} h^{-1} respectively) and longer half-lives (56.80 and 55.88 hours). However, these results differ from previous studies. Li et al. (2020) reported that the degradation of 1-nitropyrene follows a first-order kinetic decay model (Li et al., 2020). Similarly, degradation of 2-nitrofluorene might follow zero-order or second-order kinetic models (Stewart et al., 2010). These variations highlight the influence of experimental factors, microbial consortia, and ambient circumstances on degradation kinetics. Table 2 summarizes the rate constants (k) and half-lives ($T_{1/2}$) of 1-nitropyrene and 2-nitrofluorene in the presence of isolated bacterial strains and their co-inoculum.

The degradation percentage of 1-nitropyrene and 2-nitrofluorene were summarized in the Table S1. The results of the degradation experiments demonstrate that using a co-inoculum BGC01, which contains two bacterial strains: *Bacillus cereus* BG034 and *Bacillus altitudinis* BG05, improves the degradation of nitro-PAHs significantly ($p < 0.05$) throughout the experiment. BGC01 consistently outperformed each of the individual strains in the breakdown of both 1-nitropyrene and 2-nitrofluorene, indicating a synergistic effect when both strains are used together. Specifically, for 1-nitropyrene, BGC01 achieved over 46.0 % degradation within the initial 24 hours, simultaneously, strain BG034 accomplished 19.0 %, and strain BG05 attained 26.4 %. These percentages significantly rose by 48 hours, with BGC01 at 66.0 %, strain BG05 at 36.0 %, and strain BG034 at 40.0 %. The degradation percentage peaked after 72 hours, with the BGC01 scoring 76.0 %, strain BG05 49.0 %, and strain BG034 scoring 47.8 %.

In the degradation of 2-nitrofluorene strain BG034 achieved a degradation of 31.1 %, followed closely by strain BG05 at 26.1 %. However, the co-inoculum outperformed both, achieving an impressive degradation of 54.0 % at 24 hours. The BGC01's degradation level increased to 87.2 % at the 72-hour mark, significantly higher than strains BG034 (59.9 %) and BG05 (59.8 %). A control group was set up to provide a baseline for comparison with the bacterial treatments. This control comprised a PBYS medium containing 25 mg/L of nitro-PAHs devoid of bacterial inoculation. These control samples were incubated under the same conditions (37°C at 150 rpm) as the treatment groups to duplicate the experimental setting. This setting ensured that degradation observed in the treated groups could be distinctly attributed to bacterial activity instead of abiotic influences. The control group showed little degradation, with ~ 6 % for both of the nitro-PAHs (i.e. 2-nitrofluorene and 1-nitropyrene) after 72 hours, highlighting the critical role of bacterial strains in the effective degradation of nitro-PAHs. The abiotic control further demonstrated that considerable degradation occurred only in the presence of active PGPR strains. These findings substantiate that the bacterial strains and their co-inoculum possess higher capabilities in the degradation of nitro-PAHs, emphasizing their potential as efficacious bioremediation agents. Table 3 summarizes the nitro-PAHs (1-nitropyrene and 2-nitrofluorene) degradation rates of the two PGPR strains and the BGC01. Similar to the degradation percentage, the most rapid degradation rates were noted within 24 hours of incubation for 1-nitropyrene and 2-nitrofluorene. During this period, the BGC01 achieved the highest degradation rate of 1.91 mg L^{-1} h^{-1} for 1-nitropyrene and 2.25 mg L^{-1} h^{-1} for 2-nitrofluorene. Over a 72-hour duration, the BGC01 maintained the lead in degradation rates (1.05 mg L^{-1} h^{-1} for 1-nitropyrene and 1.21 mg L^{-1} h^{-1} for 2-nitrofluorene) followed closely by strains BG034 and BG05 for both compounds.

The rapid degradation of 1-nitropyrene and 2-nitrofluorene observed within the initial 24 hours of incubation is attributed to the bacteria beginning the logarithmic growth phase, during which their metabolic activity increases, resulting in the rapid use of nitro-PAHs as a carbon source. Over 76.0 % of 1-nitropyrene and 87.2 % of 2-nitrofluorene

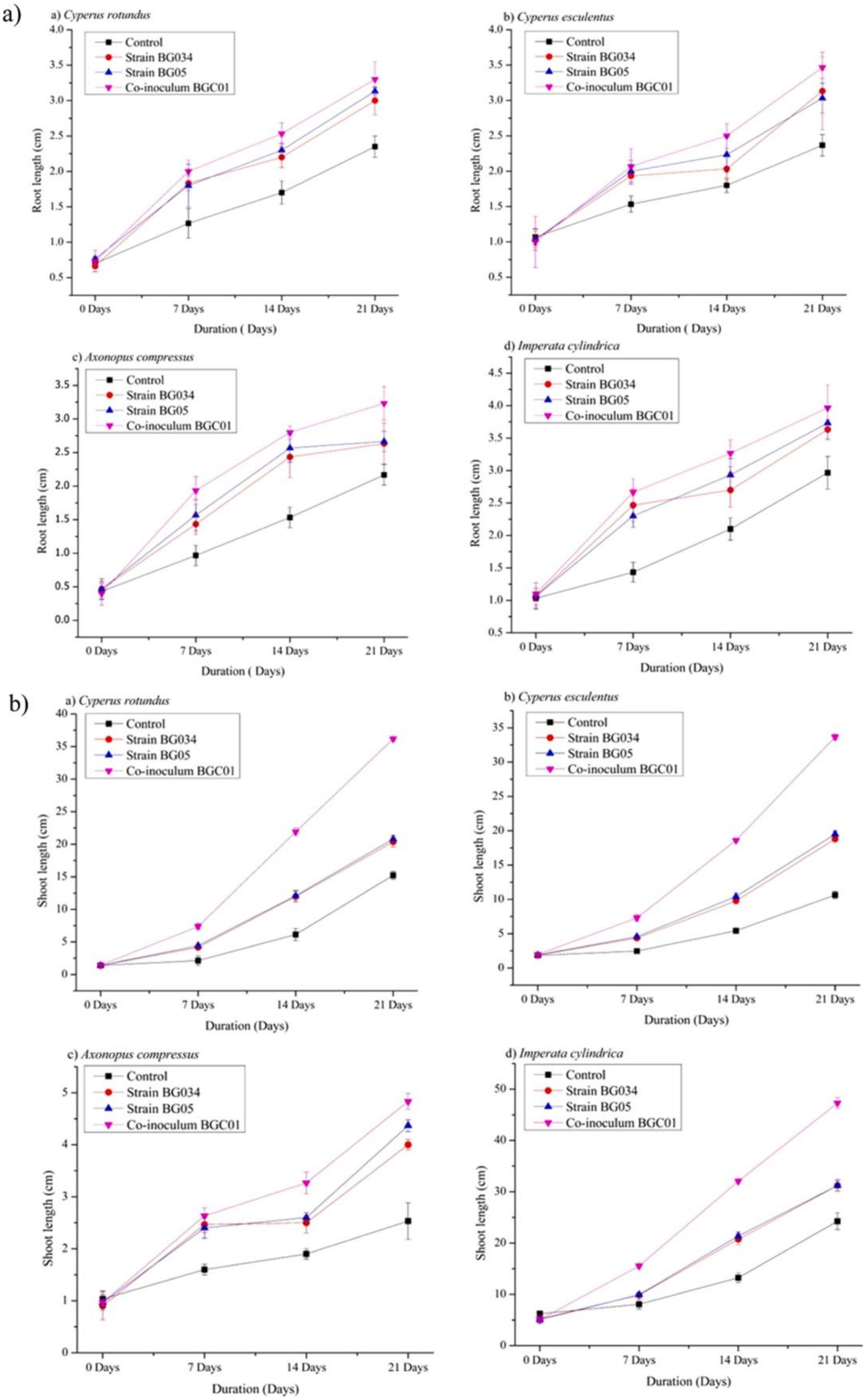

Fig. 2. Effects of bacterial inoculation on the shoot length (a) and root length (b) of *Cyperus rotundus* L. (a), *Cyperus esculentus* L. (b), *Imperata cylindrica* (L.) Beauv. (c), and *Axonopus compressus* (Sw.) P. Beauv. (d),— throughout 21 days. The study assessed the plant growth-promoting properties of isolated bacterial strains and their co-inoculum. Both individual bacterial treatments and the co-inoculum significantly increased shoot length compared to the control ($p < 0.05$), with the co-inoculum showing a notably higher enhancement than the single strains. Data are expressed as means ± S.D. (n = 3).

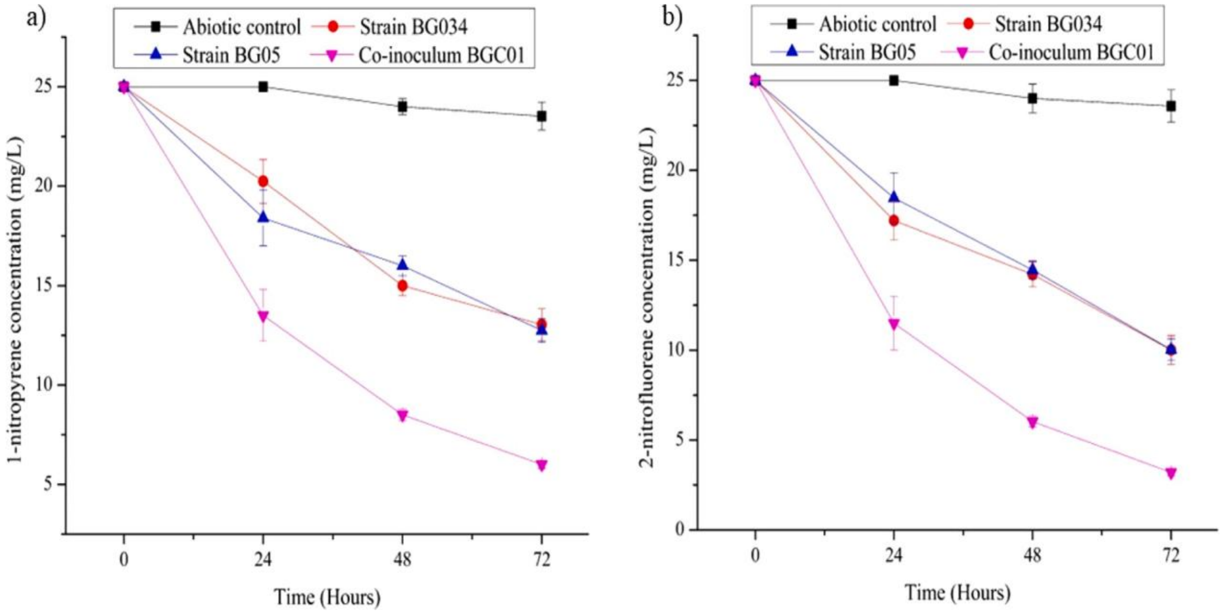

Fig. 3. Degradation of 1-nitropyrene (a) and 2-nitrofluorene (b) by formulated bacterial co-inoculum (BGC01) and individual strains (BG034 and BG05) in a shaker flask over a 72-hour incubation period. The initial concentration of each nitro-PAH was 25 mg/L, which gradually decreased with increasing incubation time. Data are presented as mean ± S.D. (n = 3) with significant differences observed at $p < 0.05$.

Table 2
Kinetic degradation model of the nitro-PAHs.

Parameter	1-nitropyrene			2-nitrofluorene		
	BG034	**BG05**	**BGC01**	**BG034**	**BG05**	**BGC01**
Regression equation	y = 0.0011x + 0.3095	y = 0.0011x + 0.3123	y = 0.0034x + 0.3063	y = —0.0122x + 3.1961	y = —0.0124x + 3.2255	y = —0.0284x + 3.1772
Coefficients of determination (R^2)	0.986	0.986	0.997	0.986	0.994	0.997
Rate constant (k) (L $mol^{-1}h^{-1}$)	0.0011	0.0011	0.0034	0.0122	0.0124	0.0284
Half-life ($T_{1/2}$) (Hours)	36.36	36.36	11.76	56.80	55.88	33.90
kinetic question	$\frac{1}{[A]} = kt + \frac{1}{[A]o}$			$Ln[A] = -kt + ln [A]_0$		

Table 3
Degradation rate (mg L^{-1} h^{-1}) of 1-nitropyrene and 2-nitrofluorene.

Time (hours)	1-nitropyrene				2-nitrofluorene			
	Abiotic Control	Strain BG034	Strain BG05	Co-inoculum BGC01	Abiotic Control	Strain BG034	Strain BG05	Co-inoculum BGC01
0–24	0	0.79 ± 0.06	1.1 ± 0.08	1.91 ± 0.05	0	1.29 ± 0.13	1.09 ± 0.12	2.25 ± 0.13
24–48	0.16 ± 0.03	0.87 ± 0.02	0.40 ± 0.03	0.83 ± 0.03	0.16 ± 0.1	0.50 ± 0.02	0.66 ± 0.01	0.91 ± 0.14
48–72	0.08 ± 0.01	0.32 ± 0.04	0.54 ± 0.02	0.41 ± 0.02	0.07 ± 0.02	0.70 ± 0.01	0.73 ± 0.02	0.47 ± 0.07
0–48	0.08 ± 0.02	0.83 ± 0.09	0.75 ± 0.03	1.37 ± 0.08	0.08 ± 0.05	0.89 ± 0.05	0.87 ± 0.06	1.58 ± 0.13
24–72	0.12 ± 0.03	0.60 ± 0.05	0.47 ± 0.01	0.62 ± 0.02	0.11 ± 0.05	0.60 ± 0.07	0.70 ± 0.01	0.69 ± 0.02
0–72	0.08 ± 0.03	0.66 ± 0.04	0.68 ± 0.06	1.05 ± 0.07	0.07 ± 0.03	0.83 ± 0.04	0.83 ± 0.05	1.21 ± 0.03

The data are expressed as mean ± S.D. (n = 3)

were degraded within 72 hours across all experimental conditions, demonstrating the significant capability of the PGPR strains for nitro-PAH degradation. The previous research highlighted the improved degrading efficiency of microbial consortia compared to single-strain. Zhong et al. (2011) reported that a consortium of *Mycobacterium* sp. APYR and *Sphingomonas* sp. PheB4 was able to degrade 50 % of 10 mg/L pyrene within a week, significantly exceeding the performance demonstrated by the individual strains (Zhong et al., 2011). Similarly, Shen et al. (2015) reported that a consortium consisting of *Pseudomonas* sp. PH1, *Bacillus* sp. PH2, *Ochrobactrum* sp. PH3, and *Pseudomonas* sp. PH4 obtained a 38 % degradation of 100 mg/L pyrene after six days (Shen et al., 2015). These studies underscore the critical role that microbial interactions play in pollution breakdown. The synergistic dynamics observed in microbial consortia can be attributed to their complementary metabolic capacities, which include one strain producing intermediates or co-factors that are then metabolized by another strain. This metabolic synergy speeds up the breakdown of complex contaminants like PAHs. The study corroborates with the findings of Wanapaisan et al. (2018), in which microbial consortia break down pyrene faster than individual strains, which was due to the division of metabolic labour and enhanced substrate utilization (Wanapaisan et al., 2018). Future investigations would endeavor to conduct comprehensive analyses to determine the significance of these differences and explore specific conditions, such as variations in substrate complexity, environmental factors, or prolonged incubation periods, which may augment the prospective synergistic advantages of co-inoculation. The previous report by Sarma et al. (2024) also highlighted that substrate availability substantially impacts the bacterial breakdown of organic contaminants such as nitro-PAHs (Sarma et al., 2024). The study underlines that the bioavailability of nitro-PAHs in the PBYS medium is critical for degradation, with bacterial breakdown efficiency proportional to substrate concentration. Bacteria require threshold concentrations of nitro-PAHs to increase and perform metabolic operations (Koner et al., 2021; Zhou et al., 2023). The substrate concentration has been proven to influence bacterial activity, which is consistent with Michaelis-Menten kinetics significantly. At minimal substrate concentrations, increased levels generally result in a corresponding enhancement of bacterial activity. Nonetheless, once the substrate concentration approaches a specific threshold, the reaction rate reaches a maximum (Vmax), indicating that the bacterial enzymes have reached saturation. Beyond this threshold, continued substrate concentration increases and decreases bacterial activity. An overabundance of a substrate may result in metabolic inhibition when hazardous byproducts accumulate, or metabolic pathways become overloaded, limiting bacterial activity. Our experimental findings revealed that 25 mg/L is the threshold concentration for bacterial activity; after which bacterial activity decreases owing to metabolic inhibition (shown in Fig. 4). These findings emphasize the need to adjust substrate concentrations for successful bioremediation, with other parameters such as contaminant complexity, medium type, and environmental conditions influencing degradation rates (Bandowe and Meusel, 2017; Vinayagam et al., 2024).

The GC-MS analysis of the BGCO1 co-inoculum-treated medium was selected because it had higher plant growth-promoting activity than the individual bacterial strains, and also had a substantial potential to remove nitro-PAHs. GC-MS analysis validated the breakdown of nitro-PAHs spiked into PBYS medium, with initial degradation into simpler hydrocarbons observed at 15 days, complete transformation into linear aliphatic hydrocarbons at 30 days with no detectable remnants of the original nitro-PAHs in the PBYS medium. This complete degradation process implies a very efficient mechanism that is assisted by the treatments. The degradation routes for nitro-PAHs observed in this study revealed a systematic conversion process involving different hydrocarbons, eventually breaking down these complex molecules into simpler linear aliphatic hydrocarbons such as decane following the application of co-inoculum, highlighting the critical function that these treatments play in the conversion of complex hydrocarbons into simpler forms (Fig. 5). For 1-nitropyrene at initial (0 days) GC-MS analysis confirmed the presence of 1-nitropyrene in the tested shaker flask. By day 15, degradation products such as naphthalene, a two-ring PAH, along with the intermediated metabolites such as 2,3,5,8-tetramethyldecane and 1-iodo-2-methylundecane, had been reported, showing that the complex structure of 1-nitropyrene has started to break down. Additional intermediate metabolites, such as methoxyacetic acid, 2-tridecyl ester, sulfurous acid, hexyl pentadecyl ester, and 6-methyloctadecane, revealed paths for methylation, esterification, and oxidative or reductive conversion processes. By day 30, advanced degradation processes had produced linear aliphatic hydrocarbons such as decane and 2,4,6-trimethyldecane, as well as substances like isoshyobunone and aromadendrene oxide-1. Similarly, for 2-nitrofluorene, the parent compound was detected at the beginning of the experiment. At 15 days, naphthalene was reported, confirming the first transformation observed in 1-nitropyrene. This finding shows that degradation processes are similar, including ring breakage and reductive de-nitration mechanisms. By day 30, the intermediate metabolites such 1H-indene, 1-methylenedodecane, and 5,8-diethyldodecane had undergone further de-aromatization and conversion into aliphatic hydrocarbon. Additional intermediates, such as 2,3,5,8-tetramethyldecane, 2,6,10,15-tetramethylheptadecane, and 2,6,10-trimethyltetradecane, etc were reported which suggested the presence of methylation, iodination, and diverse microbial metabolic processes. In both instances, the emergence of linear aliphatic compounds and methylated derivatives by day 30 underscores the considerable degradation of 1-nitropyrene and 2-nitrofluorene. This finding also supports our hypothesis that specific enzymes aid in breaking nitro-PAHs in a liquid medium. These enzymes most likely help to break down complex hydrocarbons, making them more accessible for subsequent degradation into simpler compounds. Previous studies also reported that the breakdown of 1-nitropyrene and 2-nitrofluorene by bacteria proceeded through well-defined enzymatic pathways that included nitro group reduction, ring cleavage, and mineralization and assimilation (Grifoll et al., 1992; Rafil et al., 1991). Several bacterial extracellular and intracellular enzymes, including nitroreductases, dioxygenases, and cytochrome P450 monooxygenases, have been reported on nitro-PAHs breakdown processes (Li et al., 2023). Key enzymes encompass nitroreductases, which catalyze the conversion of nitro groups to amino groups, facilitating the structural modifications necessary for subsequent degradation (Claus, 2014; Penning et al.,

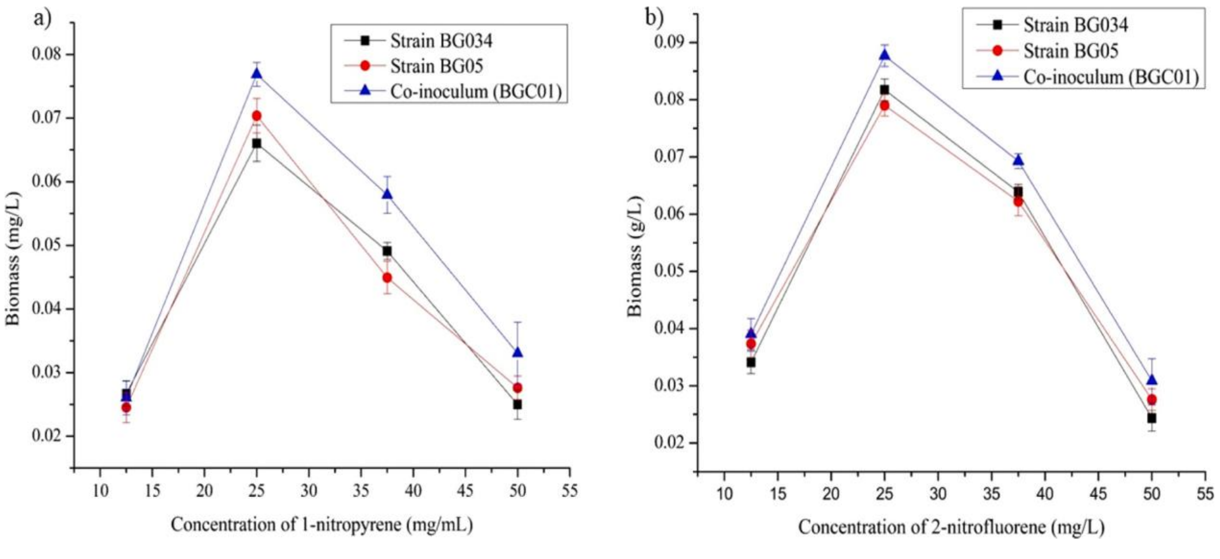

Fig. 4. Bacteria biomass (g/L) at different concentrations of 1-nitropyrene (a) and 2-nitrofluorene (b) after 7 days of incubation. The highest biomass for all individual strains and their co-inoculum was observed at 25 mg/L concentrations of 1-nitropyrene and 2-nitrofluorene. Data are expressed as means ± S.D. (n = 3).

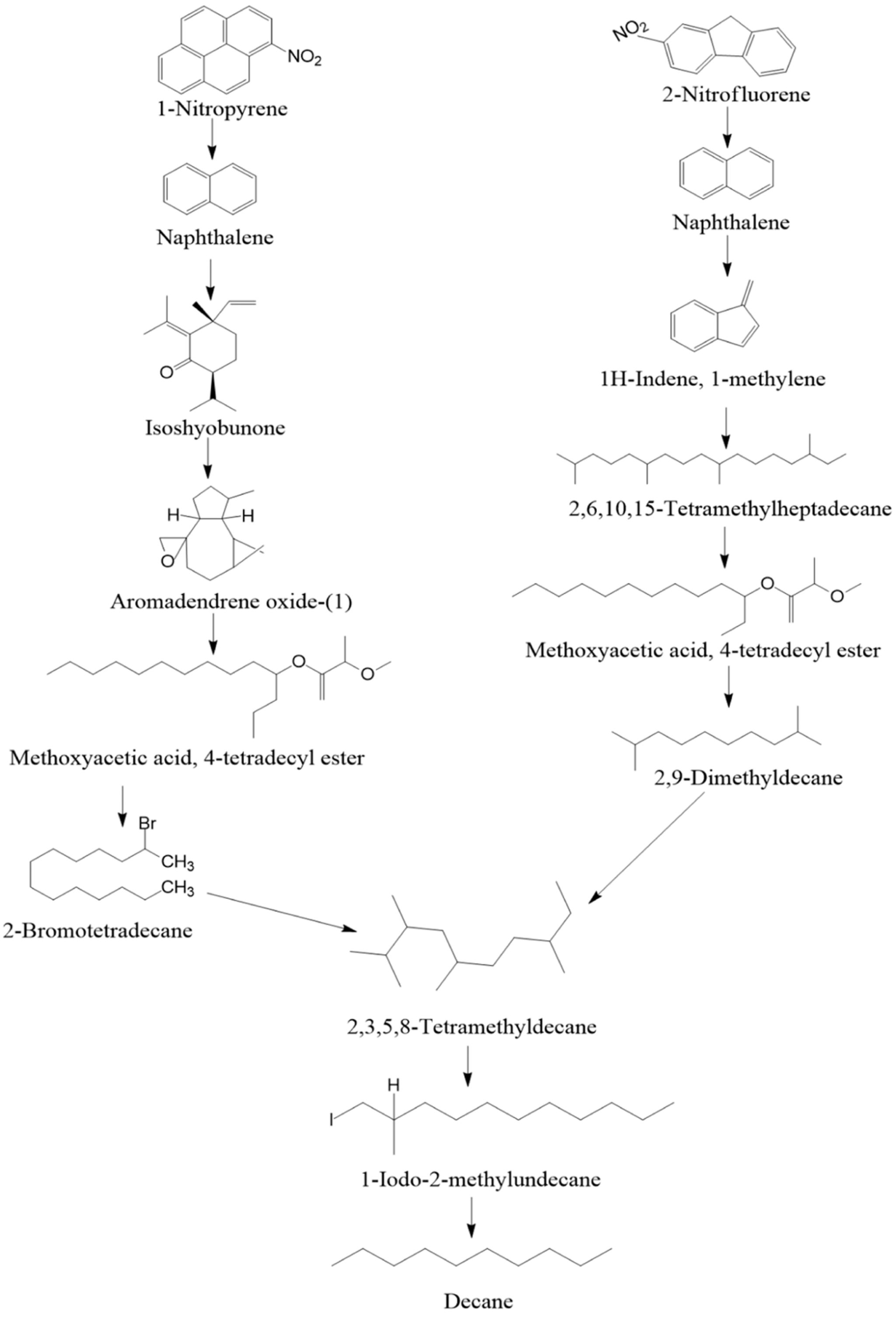

Fig. 5. Proposed nitro-PAH degradation pathways by bacterial co-inoculum in shaker flask culture.

2022). Dioxygenases, including Rieske non-heme ring-hydroxylating oxygenase (RHO) and cytochrome P450 monooxygenases (CYP450s), hydroxylate the aromatic rings, thereby improving their solubility (Sun et al., 2023). Subsequently, intradiol and extradiol dioxygenases cleave the aromatic structures, yielding intermediates such as catechol, which are then further metabolized into fatty acids, ultimately culminating in complete mineralization through the tricarboxylic acid (TCA) cycle (Das et al., 2023; Lipscomb, 2008). However, this study did not identify the specific enzymes involved in the degradation processes, highlighting the need for future investigation.

To effectively transition from laboratory findings to practical field applications, a systematic approach is essential for scaling up this technology. First, soil microcosm experiments will be conducted in a controlled greenhouse setting to evaluate the adaptability and efficiency of specific strains and their co-inoculants using contaminated soil. These experiments will help optimize the application rates of the co-inoculants and various environmental parameters to enhance both plant growth promotion and the degradation efficiency of pollutants. After successful greenhouse trials, field tests will be carried out in polluted areas. This phase will assess factors such as soil heterogeneity, microbial competition, environmental stressors, and pollutant bioavailability. Finally, to facilitate practical implementation, it is crucial to establish collaborations with environmental agencies, legislators, and the agricultural sector to integrate PGPR-based bioremediation into sustainable land management strategies. Ongoing monitoring, economic viability assessments, and environmental impact evaluations will be critical for ensuring long-term success and widespread acceptance.

4. Conclusion

This study was successful in isolating two promising nitro-PAHs tolerance PGPR strains, *Bacillus cereus* BG034 and *Bacillus altitudinis* BG05, from the rhizosphere of *Cyperus rotundus* L., *Cyperus esculentus* L., *Imperata cylindrica* (L.) Beauv., and *Axonopus compressus* (Sw.) P. Beauv., both of which exhibited plant growth-promoting properties. In greenhouse trials, the formulated co-inoculum was found to augment these effects, indicating a synergistic relationship that significantly enhanced plant shoot and root length. Furthermore, both strains, individually and in combination, successfully degraded 1-nitropyrene and 2-nitrofluorene. Notably, the co-inoculum demonstrated higher degradation efficiency than the individual strains, with 76.0 % degradation of 1-nitropyrene and 87.2 % degradation of 2-nitrofluorene after 72 hours of incubation. Future investigations should concentrate on extensive field trials and further scrutiny of the enzymatic pathways implicated in pollutant degradation, and soil heterogeneity, physicochemical differences, and microbial dynamics, all of which can influence bioavailability and degradation rates to fully capitalize on these PGPR strains' capabilities in bioremediation and sustainable agriculture. Future research should also incorporate aging protocols that simulate the long-term interactions between nitro-PAHs and soil components. Also, it should investigate approaches such as developing stress-tolerance bacterial consortia or employing biostimulants, biosurfactants, and biochar to sustain enhanced bacterial functionality in field scenarios. Furthermore, field trials should assess bioremediation technologies' long-term sustainability and environmental implications to establish the technology's scalability. Addressing these issues will allow laboratory findings to be applied and viable environmental cleanup solutions develop.

CRediT authorship contribution statement

Sarma Hemen: Conceptualization, Data curation, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Gogoi Bhoirob:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft. **Islam Nazim Forid:** Data curation, Formal analysis, Investigation, Project administration, Resources, Software, Writing – original draft.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used [https://app.grammarly.com//(License version)] to improve the readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2025.100263.

Data availability

Data will be made available on request.

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Nitro-PAHs: Occurrences, ecological consequences, and remediation strategies for environmental restoration

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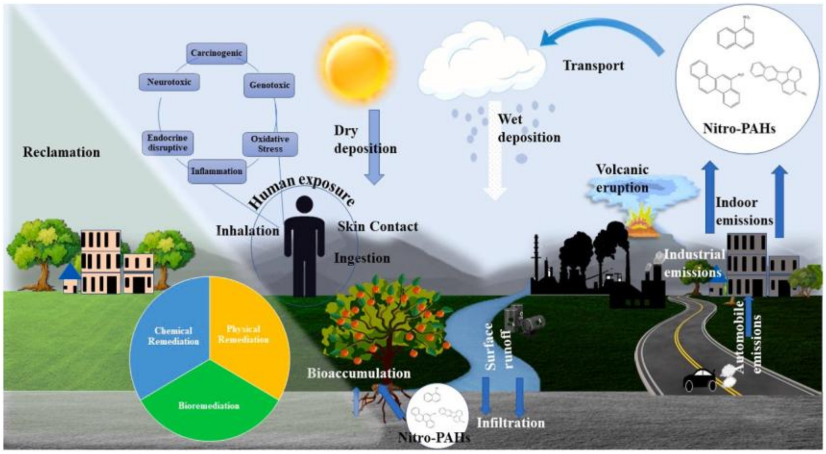
HIGHLIGHTS

- Nitro-PAHs, arising from human and natural sources, pose environmental challenges due to their persistence and long-range transport.
- Exposure through inhalation, ingestion, and skin contact raises concerns for health, including mutagenicity and carcinogenicity risks.
- Nitro-PAHs disrupt the endocrine system, leading to reproductive and developmental problems.
- Incorporating microorganisms and biostimulants enhances phytoremediation for nitro-PAHs.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are persistent pollutants that have been introduced into the environment as a result of human activities. They are produced when PAHs undergo oxidation and are highly resistant to degradation, resulting in prolonged exposure and significant health risks for wildlife and humans. Nitro-PAHs' potential to induce cancer and mutations has raised concerns about their harmful effects. Furthermore, their ability to accumulate in the food chain seriously threatens the ecosystem and human health. Moreover, nitro-PAHs can disrupt the normal functioning of the endocrine system, leading to reproductive and developmental problems in humans and other organisms. Reducing nitro-PAHs in the environment through source management, physical removal, and chemical treatment is essential to mitigate the associated environmental and human health risks. Recent studies have focused on improving nitro-PAHs' phytoremediation by incorporating microorganisms and biostimulants. Microbes can break down nitro-PAHs into less harmful substances, while biostimulants can enhance plant growth and metabolic activity. By combining these elements, the

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effectiveness of phytoremediation for nitro-PAHs can be increased. This study aimed to investigate the impact of introducing microbial and biostimulant agents on the phytoremediation process for nitro-PAHs and identify potential solutions for addressing the environmental risks associated with these pollutants.

1. Introduction

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are emerging pollutants that have gained attention due to their widespread presence in the environment and potential impact on human health and the ecosystem (Ma et al., 2023; Nowakowski et al., 2022). These compounds, characterized by nitrogen-containing functional groups, are introduced through reactions with nitrating agents (Ma et al., 2023; Nowakowski et al., 2022) Fig. 1).

Nitro-PAHs are found in air, water, soil, and even the food chain, originating from human activities and natural processes (Balmer et al., 2019). However, human sources contribute the most to their presence in the environment. These compounds can travel long distances and persist for extended periods, transported through air currents, water, and soil (Liu et al., 2017; Bandowe and Meusel, 2017). While nitro-PAHs have low water solubility and firmly attach to solid soil layers, they can be transferred to deeper soil layers through colloid-assisted transport. Their highest concentrations are typically in topsoil, gradually decreasing with increasing soil depth (Bandowe and Meusel, 2017). Consequently, nitro-PAHs pose risks to various environmental matrices, including air, water, soil, and the food chain.

Exposure to nitro-PAHs can occur through inhalation, ingestion, and skin contact. Minimizing exposure is crucial to mitigate potential health effects, which can vary based on proximity to sources, the prevalence of food and water, and indoor exposure (Gao et al., 2022; Gbeddy et al., 2022). The toxicity of nitro-PAHs remains a concern, with conflicting research findings regarding their mutagenic and carcinogenic properties (Şahin et al., 2022; Drotikova et al., 2020). Nitro-PAHs also pose a threat to aquatic life and ecosystems. They can contaminate water bodies, negatively affecting the health of aquatic organisms and potentially impacting human health (Huang and Batterman, 2014). Specific nitro-PAHs have been detected in various aquatic environments and can disrupt reproductive and developmental processes and alter the growth and survival of aquatic plants and microorganisms (Onduka et al., 2012; Lee et al., 2022).

Researchers are exploring physical, chemical, and biological remediation techniques to address the potential health and environmental impacts of nitro-PAHs. Physical methods require significant labor, while chemical approaches demand costly reagents and equipment. Biological remediation, although environmentally friendly, can be time-consuming. Among these options, using microorganisms and biostimulants shows promise for nitro-PAHs remediation. Integrating this approach with phytoremediation could enhance its effectiveness and overcome certain limitations (Wu et al., 2023).

A critical evaluation of existing literature on the role of microorganisms and biostimulants in nitro-PAHs remediation is likely the focus of ongoing research. This review would encompass previous studies investigating the use of microorganisms and biostimulants to enhance phytoremediation and assess the current understanding of the underlying mechanisms. It would also evaluate the efficacy of different microorganisms and biostimulants and any constraints or challenges associated with their utilization, ultimately providing recommendations for future research initiatives.

2. Origin and occurrences of nitro-PAHs

Nitro-PAHs originate from both natural and human sources. Natural sources include lightning strikes, volcanic eruptions, and meteor impacts, where nitro-PAHs are synthesized from PAH precursors (Bandowe and Meusel, 2017; Li et al., 2015). Biological processes, such as nitrification in soil and water, can also lead to nitro-PAHs formation from PAH precursors (Strandberg et al., 2023; Zimmermann et al., 2013). However, the majority of nitro-PAHs present in the environment today are a result of human activities.

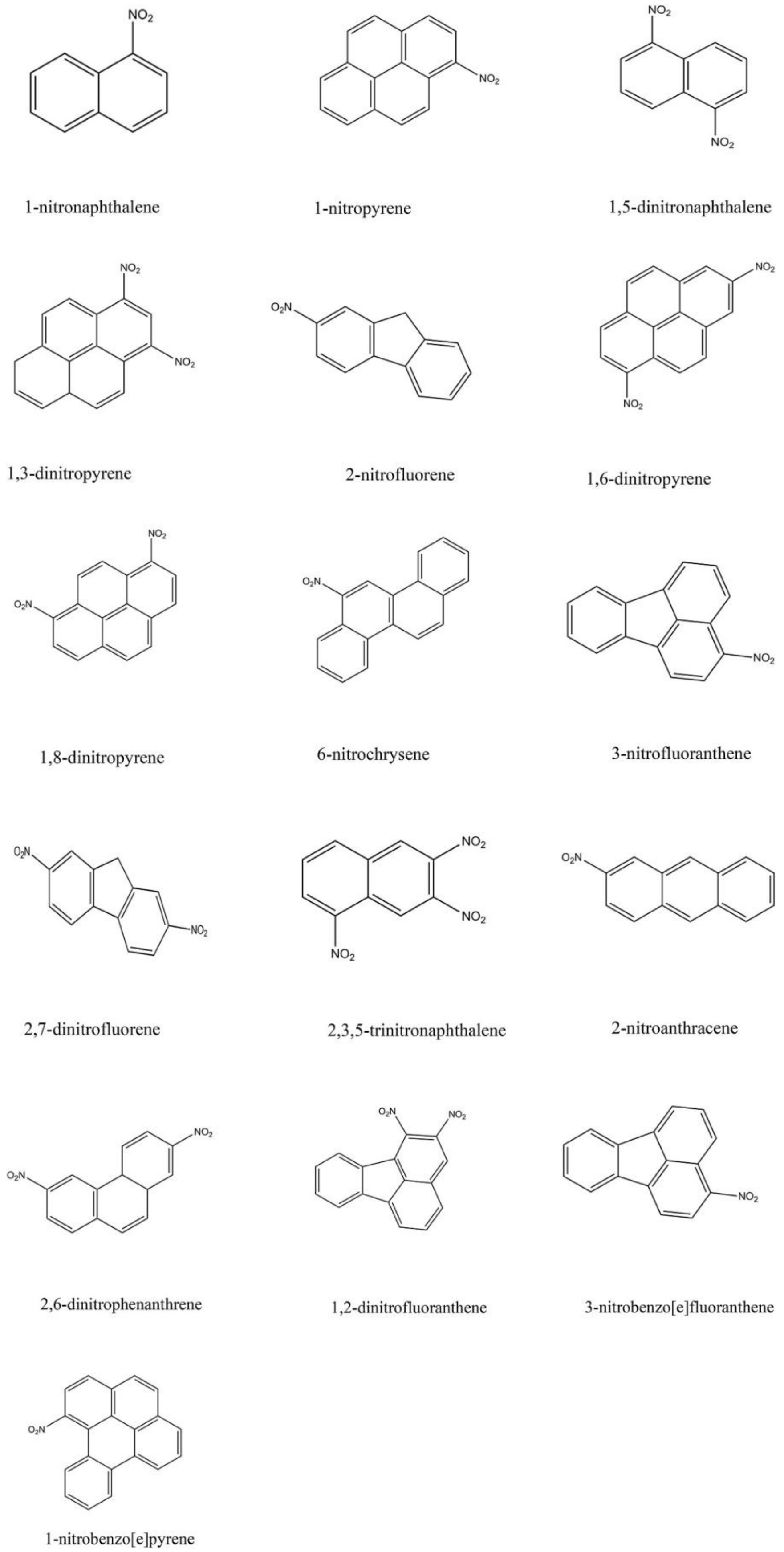
Anthropogenic sources of nitro-PAHs include the combustion of fossil fuels, such as coal, oil, and natural gas, and nitrogen oxide (NOx) emissions from vehicles and industrial processes (Ozaki et al., 2010). Nitro-PAHs are formed during combustion by interacting with PAH precursors and nitrogen oxides (NOx). Nitrogen oxides, often generated while burning fossil fuels, facilitate the functionalization of PAHs to form nitro-PAHs (Ma et al., 2023; Möller et al., 1993). Various factors, including the type of fuel, combustion conditions, and the presence of other contaminants, influence this process, which occurs at high temperatures.

Emissions from automobiles and industrial activities are significant sources of environmental nitro-PAHs. Nitro-PAHs formation occurs through the emission of nitrogen oxides from the exhaust of gasoline-powered vehicles and diesel engines (Idowu et al., 2019). Industrial processes, such as those in boilers, power plants, and other facilities, also contribute to the emission of nitrogen oxides and the subsequent formation of nitro-PAHs. Additionally, the use of nitrogen-based fertilizers can result in the formation of nitro-PAHs. Applying these fertilizers to crops and soil leads to nitrification, where microorganisms decompose the fertilizers and produce nitrite and nitrate ions. These ions can react with PAH precursors, forming nitro-PAHs in the soil (Mackiewicz-Walec and Krzebietke, 2020). The occurrence of nitro-PAHs within the environmental matrix is summarized in Table 1.

3. Characteristics of nitro-PAHs

The properties of nitro-PAHs are related to their molecular structure, including a nitro functional group (NO_2) and the aromatic ring characteristic of PAHs. The nitro group confers distinct chemical properties to nitro-PAHs compared to PAHs, significantly affecting their environmental behavior and toxicity. The following are the physicochemical characteristics of nitro-PAHs:

The solubility of nitro-PAHs is affected by several factors, including the individual nitro-PAH's chemical structure and physical characteristics and the solvent's qualities. Nitro-PAHs are more soluble in organic solvents like benzene and toluene than in water (Stewart et al., 2010). For example, 1-nitronaphthalene is soluble in benzene, chloroform, and carbon disulfide. 1-nitropyrene, on the other hand, is soluble in benzene, ethanol, diethyl ether, toluene, and tetrahydrofluorenone (Chan, 1993). 2-nitrofluorene is soluble in benzene and acetone, and 6-nitrochrysene is soluble in ethanol, nitrobenzene, carbon disulfide, diethyl ether, benzene, and acetic acid (Nascimento et al., 2019; Sun et al., 2020). However, the solubility of these compounds in water is generally low. At a temperature of 25 °C, the solubility of 1-nitronaphthalene in water is 9.18 mg/L, while the solubility of 1-nitropyrene in water is 1.18×10^{-2} mg/L (Gang and Xiaobai, 1992; Nascimento et al., 2019). These solubility values indicate that these compounds are relatively water-insoluble and more likely to dissolve in organic solvents. The solubility of nitro-PAHs can also be altered by pH, temperature, and the presence of other compounds in the environment. The solubility of nitro-PAHs in water diminishes with increasing molecular size, which might lead to the development of more persistent and hazardous species in the environment. Indeed, the solubility characteristic of nitro-PAHs has a substantial influence on their transportation and distribution in the environment, their accessibility to organisms, and their potential for damage in the environment.



1-nitronaphthalene
1-nitropyrene
1,5-dinitronaphthalene
1,3-dinitropyrene
2-nitrofluorene
1,6-dinitropyrene
1,8-dinitropyrene
6-nitrochrysene
3-nitrofluoranthene
2,7-dinitrofluorene
2,3,5-trinitronaphthalene
2-nitroanthracene
2,6-dinitrophenanthrene
1,2-dinitrofluoranthene
3-nitrobenzo[e]fluoranthene
1-nitrobenzo[e]pyrene

Fig. 1. Chemical structure of some specific nitro-PAHs.

Nitro-PAHs have a wide range of vapor pressures, which is highly determined by their molecular structure, especially the number of benzene rings and the presence of nitro groups. The number of benzene rings has an inverse relationship with vapor pressure, which is defined as the equilibrium pressure exerted by a gaseous substance in equilibrium with its condensed phases (solid or liquid) at a given temperature (Speight, 2017, 2020). For example, 1-nitronaphthalene, which has two rings, has a vapor pressure of 0.0154, whereas 1-nitrofluorene, which has three rings, has a vapor pressure of 9.7×10^{-5}, and 2-nitrofluoranthene, which has four benzene rings, has a vapor pressure of 9.9×10^{-7} (Kielhorn et al., 2003). The addition of a nitro group to PAHs reduces their vapor pressure in contrast to nitro-free counterparts due to increased molecular polarity, making the molecule less volatile (Barrado et al., 2013). For example, the vapor pressure of pyrene is 6.0×10^{-4}, but with the addition of one nitro group (1-nitropyrene), it decreases to 4.4×10^{-6}. Similarly, Naphthalene has a vapor pressure of 10.4, but 2-nitronaphthalene, with one extra nitro group, has a vapor pressure of 3.2×10^{-2} (Kielhorn et al., 2003). This lowered volatility has a major impact on nitro-PAHs atmospheric behavior, influencing parameters such as transit, dispersion, and environmental persistence (Hayakawa, 2022; Hussain et al., 2019). The role of temperature and pressure in determining nitro-PAHs vapor pressure is critical, as these factors influence the equilibrium between gaseous and condensed phases, with vapor pressure generally increasing with temperature for most substances, albeit with variations depending on the specific molecular structure of nitro-PAHs (Abdel-Shafy and Mansour, 2016).

Nitro-PAHs have higher boiling points than PAHs, owing to intermolecular forces that regulate molecular interactions (Idowu et al., 2019). Van der Waals forces, which include dispersion forces, dipole-dipole interactions, and hydrogen bonding, have a significant impact on the boiling points (Chakarova and Schröder, 2005). Temporary changes in electron density inside molecules provide dispersion forces, which attract surrounding molecules (Cortés-Arriagada, 2021; Ikawa et al., 2021). Larger nitro-PAHs have higher boiling points due to larger dispersion forces. Dipole-dipole interactions, which are present in nitro-PAHs with polar bonds (for example, in the nitro group), contribute to total molecule polarity, influencing intermolecular attractions and boiling temperatures (Eiroa et al., 2010). Because of greater dipole-dipole interactions, nitro-PAHs with more polar functional groups may have higher boiling points. While hydrogen bonding is less prevalent in nitro-PAHs than in compounds containing hydroxyl or amino groups, its presence can affect boiling points. Due to the formation of weak hydrogen bonds between molecules, nitro-PAHs with hydrogen bond donor or acceptor sites may have somewhat higher boiling points. The boiling points of nitro-PAHs are also influenced by their molecular weight and mass (Şahin et al., 2022). Increased size and mass provide stronger van der Waals forces and total molecular attraction, resulting in higher boiling points. Specific nitro-PAHs such as 1-nitropyrene, 1,3-dinitropyrene, and 1,2,4-trinitrofluoranthene are examples of this since extra nitro groups lead to greater boiling points (Kielhorn et al., 2003). For example, 1-nitropyrene has a boiling point of 390.29 °C, but 1,3-dinitropyrene has a higher boiling point of 434.19 °C due to an additional nitro group. The addition of extra nitro groups to 1,2,4-trinitrofluoranthene raises its boiling point to 615.240 °C, suggesting a stronger intermolecular force (Kielhorn et al., 2003). Similarly, comparing 2-nitroanthracene to 1-nitropyrene reveals that the former, with an extra nitro group, has a higher boiling point of 423.9 °C, suggesting stronger intermolecular forces (IARC, 2014).

Furthermore, nitro-PAHs can undergo several reactions in soil settings, including oxidation, protonation, reduction, and complexation (Cao et al., 2022; Chu et al., 2023). These transformations could lead to

Table 1
The concentrations of nitro-PAHs in various environmental matrices.

Environmental matrix	The concentration of nitro-PAHs (Σ nitro-PAHs)	Number of nitro-PAHs	Analysis technique	Location	Reference
Soil	0.4–4.6 ng g^{-1}	4	GC-MS,	Surface soil samples of Yangtze River Delta, China	Cai et al. (2017)
	396–2530 ng g^{-1}	15	GC-MS	Kathmandu, Pokhara, Birgunj, and Biratnagar of Nepal	Yadav and Devi (2021)
	29–158 ng g^{-1}	11	HPLC	Pham Van Dong, Hanoi, Vietnam	Pham et al. (2015)
	29–158 ng g^{-1}	11	GC-MS	Surface soils of Xi'an, central China	Wei et al. (2015)
	4–5.2 ng g^{-1}	9	HPLC	Road side soil of Catania, Italy	(Gudi et al., 2012)
	3.61–5.12 ng m^{-3}	10	HPLC	Haidian, China	Zhang et al. (2020)
	0.31 ± 0.23 ng g^{-1}	18	GC-MS	Kosetice, Europe	Wietzoreck et al. (2022)
Water	604 ng L^{-1}	5	HPLC	Asano River, Japan	Chondo et al. (2013)
	14.7–235 ng L^{-1}	15	GC/TSQ quantum triple quadrupole tandem mass spectrometry	Taige Canal, Changzhou City, China	Kong et al. (2023)
	19.7 ng L^{-1}	6	Purge-assisted headspace solid-phase microextraction combined with gas chromatography/negative ion chemical ionization mass spectrometry	Luchuan River, China	Hung et al. (2012)
Air	26.1 ± 25.9 ng m^{-3}		GC-MS	Bhagwan Talkies crossing, Agra, India	Verma et al. (2022)
	0.078–11.7 ng m^{-3}	5	GC-MS	Birmingham, United Kingdom	Alam et al. (2015)
	0–0.001 ng m^{-3}	5	GC-MS	Oregon, USA	Lafontaine et al. (2015)
	0.025–0.72 ng m^{-3}	13	HPLC with chemiluminescence	Chiang Mai, Thailand	Chuesaard et al. (2014)
	0.5–7 ng m^{-3}	12	GC-MS	Xi'an, China	Bandowe et al. (2014)
	2.26–5.86 ng m^{-3}	6	GC-ECD	Athens, Greece	Tsakas et al. (2010)

Table 2

Physicochemical properties of nitro-PAHs (Adopted from (Kielhorn et al., 2003)).

Sl. No	Nitrated polycyclic aromatic hydrocarbons	Molecular formula	Molecular weight (g/mol)	Color	Boling Point (°C)	Malting Point (°C)	Vapor Pressure [mmHg]	Density (20 °C)
1	1-nitronaphthalene	$C_{10}H_7NO_2$	173.17	Pale yellow solid	304–305 °C	61 °C	0.00048	1.33
2	1-nitropyrene	$C_{16}H_9NO_2$	247.25	Yellow solid or gold solid	390.29 °C	155 °C	0.00000006	1.16
3	1,5-dinitronaphthalene	$C_{10}H_6N_2O_4$	218.17	Yellowish-white or light-yellow solid or light-yellow crystalline powder	358.84 °C	138–141 °C	0.00000428	1.58
4	1,3-dinitropyrene	$C_{16}H_8N_2O_4$	292.24	Yellow-orange crystalline solid	434.19 °C	274–276 °C		1.28
5	2-nitrofluorene	$C_{13}H_9NO_2$	211.22	Cream-colored solid	350.9 °C	158 °C	0.00000954	1.18
6	1,6-dinitropyrene	$C_{16}H_8N_2O_4$	292.24	Yellow-colored solid	434.19 °C	300 °C	0.0000000091	1.28
7	1,8-dinitropyrene	$C_{16}H_8N_2O_4$	292.24	Yellow to orange-colored solid	434.19 °C	>300 °C	0.0000000091	1.28
8	6-nitrochrysene	$C_{18}H_{11}NO_2$	273.36	Yellow, orange-yellow, or chrome-red crystalline solid	416.31 °C	215 °C	0.00000001	1.21
9	3-nitrofluoranthene	$C_{16}H_9NO_2$	247.25	Pale yellow to light brown crystalline solid.	390.29 °C	157–159 °C	0.000000104	1.16
10	2,7-dinitrofluorene	$C_{13}H_8N_2O_4$	256.21	Yellow crystalline solid	399.45 °C	330–334 °C	0.00000019	1.32
11	2,3,5-trinitronaphthalene	$C_{10}H_5N_3O_6$	263.16	Yellow crystalline solid	286.00–287.00 °C	160–162 °C	0.004000	1.72
12	2-nitroanthracene	$C_{14}H_9NO_2$	223.23	Yellow-colored solid	423.9 °C	232.76		1.31
13	2,6-dinitrophenanthrene	$C_{14}H_8N_2O_4$	268.22	Yellow to orange crystalline solid	489.03	207.48		1.61
14	1,2-dinitrofluoranthene	$C_{16}H_8N_2O_4$	292.246	Yellow crystalline solid.	548.3 ± 23	279.12	0.00000000091	1.61
15	1,2,4-trinitrofluoranthene	$C_{16}H_7N_3O_6$	337.24	Light brown crystalline solid	615.2 ± 40.0 °C	305.13 °C		1.71
16	3-nitrobenzo[e] fluoranthene	$C_{20}H_{11}NO_2$	297.3	Orange crystals solid	533.5 ± 19.0 °C	211–212 °C	0.00000000031	1.41
17	1-nitrobenzo[e]pyrene	$C_{20}H_{11}NO_2$	297.3	Orange crystals solid	533.5 ± 19.0 °C	250–250.5 °C	0.0000000011	1.30

the generation of secondary reactive derivatives of nitro-PAHs, which might exhibit altered properties compared to the parent compounds. These derivatives might possess different chemical reactivities, solubilities, or environmental fates, impacting their persistence and potential environmental risks. When evaluating the possible influence of nitro-PAHs on human health and the environment, it is critical to consider their reactivity. Their toxicity, persistence, and environmental movement can be affected by their capacity to perform chemical reactions. Furthermore, their reactivity influences their ability to produce secondary products, such as nitroso and nitro derivatives, which may have different characteristics and effects than the parent compounds. Below Table 2 presents an exhaustive list of nitro-PAHs' physicochemical properties clearly and concisely.

4. Analytical techniques used to monitor the nitro-PAHs in air, soil, and water

Nitro-PAH monitoring in air, soil, and water demands a complex strategy that combines extraction, detection, and quantification approaches (Bandowe and Meusel, 2017). Due to their typically lower concentrations across environmental compartments and their lower sensitivity to electron impact (EI) ionization, more than 90% of nitro-PAHs analytical methods depend on gas chromatography (GC) in conjunction with mass spectrometry (MS) and high-performance liquid chromatography (HPLC) in conjunction with either fluorescence detection or mass spectrometry (Sun et al., 2020). The three phases of nitro-PAHs in air samples are analyzed: vapor, total suspended particles, and particular size fractions such as PM1, PM2.5, and PM10. Polyurethane Foams (PUFs) and quartz filters are commonly used for collecting nitro-PAHs in the vapor phase and particulate matter, respectively. Following the collection, the PUF and quartz filter are carefully wrapped in aluminum foil to prevent sample photolysis, then stored at —20 °C (Mulder et al., 2019; Wang et al., 2022). During water sample collection, dissolved and particle phases are separated through filtration. A stainless-steel grab sampler is used for sediment recovery, with depths ranging from 0 to 5 cm being targeted. After collection, sediment samples are well mixed before being put in stainless steel glass vials and preserved at —20 °C. Concurrently, water samples from 0.5 m deep are preserved at 4 °C in 2 L amber glass jars (Kong et al., 2023). Surface soils (0–20 cm, A-horizon) and deeper soil layers have been sampled systematically. The soil samples are packed into pre-cleaned aluminum foil bags throughout the collection procedure. Following that, the soil samples are air-dried before being ground and sieved (2 mm mesh size). These soil samples are then kept at —20 °C to ensure sample integrity until they are analyzed further (Ma et al., 2023; Sun et al., 2017). Following sample collection, nitro-PAHs present in solid materials like soils, sediments, and plant matter, and those concentrated on solid phases are commonly extracted using Soxhlet extraction, QuEChERS, automated shaking, pressured liquid extraction, microwave extraction, supercritical fluid extraction, and ultrasonication (Eiroa et al., 2010; Jing et al., 2023). Solvents such as dichloromethane (DCM), methanol, acetone, and their combinations, such as acetone/dichloromethane and methanol/acetone, are used (Bandowe and Meusel, 2017). The selection of technique and solvent is influenced by parameters such as sample matrix, targeted chemicals, and subsequent analytical needs. The qualitative and quantitative analysis of nitro-PAHs requires a variety of mass spectrometry techniques, including GC–NCI–MS, GC-ECD, GC-MS with electron ionization (EI), HPLC-MS with particle beam interface, HPLC-GC with atomic emission detector, ion trap mass spectrometry, TOF-MS, and HPLC-MS with electrochemical cell (Kawanaka et al., 2007; Lee et al., 2022). As mass analyzers, QMS, ToF, and Orbitrap are also used (Lee et al., 2022). Due to the weak fluorescence signals of nitro-PAHs, electrochemical and fluorescence detectors are limited. Advances in analytical instrumentation have resulted in the development of alternative techniques such as gas chromatography with atmospheric pressure chemical ionization (GC-APCI) and comprehensive two-dimensional gas chromatography (GCGC), which have increased the capabilities of nitro-PAHs analysis in complex environmental matrices (Galmiche et al., 2021).

5. Ecological consequences of nitro-PAHs

One of the most severe ecological consequences of nitro-PAHs is their toxicity to organisms. These compounds have been demonstrated to pose risks to various species, including aquatic organisms, terrestrial invertebrates, and mammals (Onduka et al., 2012). Nitro-PAHs can potentially induce genetic mutations and DNA damage, oxidative stress, endocrine disruption, neurotoxicity, and other health leading to reduced fitness and population declines (Bandowe and Meusel, 2017; Huang et al., 2014). Some nitro-PAHs have also been recognized as likely human carcinogens, emphasizing their potential threats to animal and human health. Table 3 provides a summary of the health impact associated with nitro-PAH exposure. The toxicological consequences of nitro-PAHs, including their possible influence on human health and the environment, are described below.

5.1. *Carcinogenic effects of nitro-PAHs*

According to research studies conducted by Wo´jcik et al. (2022), nitro-PAHs have been identified as a potential threat to human health due to their carcinogenic properties. These compounds have been classified as carcinogenic to humans based on evidence from laboratory animal studies and epidemiological studies. Nitro-PAHs' capacity to generate genetic alterations and DNA damage is thought to be responsible for their carcinogenic effects (Claxton, 2015). Furthermore, certain nitro-PAHs are metabolized to other chemicals that are much more genotoxic, increasing the potential for carcinogenic consequences (Wo´jcik et al., 2022). The International Agency for Research on Cancer (IARC) has categorized a variety of nitro-PAHs based on their propensity to cause cancer in experimental animals and humans. The most worrying are 3-nitrobenzanthrone and dinitropyrenes, which have been recognized as Group 1 carcinogens by the IARC. This implies that there is adequate evidence to show that these substances can cause cancer in humans (Arlt, 2005; Linhart et al., 2012). Other nitro-PAHs have been classed as Group 2A, 2B, or 3 carcinogens based on their ability to induce cancer in humans. For example, 1-nitropyrene and 6-nitrochrysene are categorized as Group 2A carcinogens, likely to cause human cancer (Su et al., 2022; Sun et al., 2009). 2-nitofluorene, 1,6-dinitropyrene, and 1, 3-dinitropyrene are classified as Group 2B carcinogens, suggesting they may cause human cancer. Finally, 1-nitronaphthalene is classed as a Group 3 carcinogen, which means there is inadequate evidence to classify it as a human carcinogen (WHO, 2023).

Among the nitro-PAHs, 3-nitrobenzanthrene extensively studied compounds of this group for its carcinogenic properties. Landvik et al. (2010) studied the molecular mechanism behind nitro-PAH's carcinogenic effects. They concentrated on the chemical 3-nitrobenzanthrene and its metabolites, looking at how they affect DNA damage and cell signaling pathways in mouse hepatoma Hepa1c1c7 cells. They discovered that 3-nitrobenzanthrene induced cell death and severe DNA damage, including the production of DNA adducts, single-strand breaks, and oxidative DNA lesions. The chemical also phosphorylated p53, chk1, chk2, H2AX, ATM, and MAPKs. Furthermore, 3-nitrobenzanthrene induced apoptosis by translocating Bax to mitochondria and activating p53, whereas 3-aminobenzanthrene largely influenced the immune system by boosting IL-6 production. These findings shed light on the molecular processes behind the mutagenic and carcinogenic effects of 3-nitrobenzanthrene and its metabolites (Landvik et al., 2010).

Hansen et al. (2007) study also sheds light on the carcinogenic potential of 3-nitrobenzanthrene emphasizing the relevance of elevated intracellular ROS levels in fostering an environment susceptible to DNA damage and cancer progression. Their findings emphasize the critical function of elevated ROS inside cells in promoting DNA damage and contributing to cancer development (Hansen et al., 2007; Lamy, 2004).

Chen et al. (2000) and Iwanari et al. (2002) conducted in vitro studies on the metabolism of 6-nitrochrysene, a carcinogenic nitro-PAH, which included particular cytochrome P450 enzymes. CYP1A2 was discovered as the enzyme responsible for the oxidation of 1,2-dihydroxy-6-nitrochrysene in the human liver, whereas CYP1A1 had a role in the same oxidation process in the human lung (Chen et al., 2000; Iwanari et al., 2002). Furthermore, CYP3A4 was discovered to enhance the

Table 3
The health impact of specific nitro-PAHs.

Specific nitro-PAHs	Occurrence	Carcinogenic group	Exposure route	Associated disease	Reference
1-nitronaphthalene	Engine exhaust, photocopy machines, and the Petroleum industry	Group 3	Inhalation	Coughing, acute lungs and liver infection, wheezing, irritation, redness, itching, nausea, vomiting, and diarrhea.	(Lin et al., 2009; Verschoyle et al., 1993)
1-nitropyrene	Diesel engines, gasoline engines, aircraft exhaust, and tobacco smoke	Group 2A	Inhalation	Adenocarcinoma, asthma, chronic obstructive pulmonary disease, emphysema, atherosclerosis, and hypertension	(Yarmohammadi and Karimi, 2024; Gao et al., 2022)
1,5-dinitronaphthalene	Diesel engines, gasoline engines, aircraft exhaust, and tobacco smoke	None carcinogen	Inhalation	Methemoglobinemia, eye irritation, skin irritation, respiratory irritation, kidney degeneration, and kidney damage	IARC (2014)
1,3-dinitropyrene	Diesel and Gasoline Engine Exhausts and kerosene heaters	Group 2B	Inhalation	Liver neoplasms, histiocytoma, papilloma, and mammary Neoplasms	Misaki et al. (2022)
2-nitrofluorene	Diesel exhaust and cigarette smoke	Group 2 B	Inhalation and skin contact	Irritation, inflammation, endocrine-disrupting, and liver neoplasms	Zhou et al. (2022)
1,6-dinitropyrene	Diesel and gasoline engines, kerosene heaters, and photocopy machines	Group 2 B	Inhalation and skin contact	Histiocytoma, Papilloma, vulvar neoplasms, and Papilloma	IARC (2014)
6-nitrochrysene	Coal tar, crude oil, fossil fuels, and diesel exhaust	Group 2 B	Inhalation and skin contact	Liver neoplasms, cell transformation, and mammary neoplasms	(IARC, 2014; Zhang et al., 2023)
3-nitrofluoranthene	Diesel exhaust and cigarette smoke	Group 3	Inhalation and skin contact	Neoplastic, apoptosis, and necrosis	(Asare et al., 2009; Chlebowski et al., 2017)
2,7-dinitrofluorene	Diesel and Gasoline engine exhausts	Group 2A	Inhalation	Eye, skin, and respiratory tract irritation and develop cancer	Malejka-Giganti et al. (2008)

reduction of 6-nitrochrysene to 6-aminochrysene. The activation of CYP1A1 protein and mRNA expression by 6-nitrochrysene in human hepatoma HepG2 cells highlighted the relevance of assessing the liver's metabolic capability. Furthermore, 6-nitrochrysene stimulated CYP1A1 mRNA production in human lung carcinoma NCI–H322 cells, and induction of multiple CYP genes, including CYP1A1, CYP1A2, and CYP1B1, was observed in various cell lines derived from different human tissues (Chen et al., 2000).

5.2. *Genotoxicity of nitro-PAHs*

Nitro-PAHs have been proven to produce genotoxicity, including mutations, chromosomal abnormalities, and DNA damage, which raises the possibility of contributing to cellular dysfunction, altered signaling pathways, disturbed homeostasis, and, ultimately, the manifestation of numerous toxic consequences (Landvik et al., 2007; Rosenkranz and Mermelstein, 1985). Nitro-PAHs are metabolically activated and produce reactive intermediates that can covalently bind to DNA, creating DNA adducts that prevent DNA replication and transcription, which play a crucial role in induced cancer (Fu et al., 1994; Penning et al., 2022). Additionally, nitro-PAHs can induce oxidative stress by generating reactive oxygen species, causing lipid peroxidation, and DNA damage, including DNA strand breakage, oxidation to 8-oxo-2′-deoxyguanosine, and DNA-adducts (Fu et al., 2012). Cells engage DNA repair mechanisms to reverse the damage caused by nitro-PAHs (Arlt, 2005). However, mutations may occur if these mechanisms become overloaded (Landvik et al., 2007). Previous studies have demonstrated that nitro-PAHs can induce mutations in bacteria and in vitro studies conducted with mammalian cells. For example, Shane et al. (1991) reported that 3-nitrofluoranthene, 1,2-dinitrofluoranthene, and 1,3-dinitrofluoranthene were potent mutagens in *Salmonella typhimurium* TA100 and TA1535 strains (Shane et al., 1991). Furthermore, Abdullahi and Karami-Mohajeri (2014) found that 1-nitropyrene caused gene mutations in various cell lines, including human diploid fibroblasts, a human hepatoma-derived cell line, mouse lymphoma cells, and Chinese hamster ovary cells (Abdollahi and Karami-Mohajeri, 2014). The genotoxic effects of nitro-PAHs are believed to result from their ability to generate ROS and cause oxidative stress, leading to DNA damage and other cellular components. Moreover, Claxton (2014) and Krejčová-Širlová et al. (2023)highlighted that nitro-PAHs can bind to DNA and form adducts, resulting in direct damage to genetic material and an increased risk of mutations.

5.3. *Oxidative stress and inflammation*

Nitro-PAHs have been shown to cause oxidative stress, a condition characterized by an imbalance between ROS production and the cells' antioxidant defense mechanisms (Lahiri, 2022). Oxidative stress may harm cellular components, including lipids, proteins, and DNA, and is thought to play a part in several diseases like cancer, cardiovascular disease, and neurological disease (Pizzino et al., 2017; Uttara et al., 2009). The oxidative stress generated by nitro-PAHs might be caused by the chemicals directly or by the chemicals' conversion to more reactive molecules. Furthermore, nitro-PAHs have been demonstrated to promote inflammation, which is the immune system's reaction to damage or infection. Inflammation can damage tissue and lead to chronic and acute infections. Shang et al. (2017) evaluated the capacity of 1-nitropyrene and 3-nitrofluoranthene to activate the Nrf2/ARE antioxidant defense system, as well as the role of the PI3K/Akt pathway in regulating pro-inflammatory responses in A549 cells. They propose that 1-nitropyrene and 3-nitrofluoranthene can activate the Nrf2/ARE system and that the PI3K/Akt pathway may control pro-inflammatory responses (Shang et al., 2017). Øvrevik et al. (2010) reported that three particular nitro-PAHs 1-nitropyrene, 3-nitrofluoranthene, and 3-nitrobenzanthrone, have a substantial role in inducing inflammation in human. These substances have been proven to cause oxidative stress and activate pro-inflammatory pathways, increasing inflammation and potentially harmful health consequences (Øvrevik et al., 2010). Considerable research has been conducted to investigate the cytotoxic and genotoxic effects of nitro-PAHs on living organisms. However, comparatively less emphasis has been placed on studying the inflammatory properties of these substances. This indicates the need for further research to comprehensively understand the pro-inflammatory potential of nitro-PAHs and their potential implications for human health.

5.4. *Endocrine disruption*

Nitro-PAHs have been demonstrated to have endocrine-disruptive properties. Exposure to nitro-PAHs can affect hormone levels and disrupt normal hormone signaling, resulting in various impacts such as alterations in growth and development, fertility, and metabolism (Kameda et al., 2011). Some nitro-PAHs can also bind to and activate the estrogen receptor, resulting in estrogenic effects and the development of hormone-sensitive malignancies like breast cancer. Endocrine disruptive action is well documented for 2-nitrofluorene, 3-nitrofluoranthene, 1, 3-dinitropyrene, 6-nitrochrysene, 8-nitrofluoranthene, 1,6-dinitropyrene, and 1,8-dinitropyrene (Misaki et al., 2022; Zhou et al., 2022).

It is crucial to highlight that nitro-PAHs' toxicity can be impacted by various factors, including the structure of nitro-PAHs, the route and length of exposure, and the sensitivity of the target cells or tissues. Also, coexisting with polychlorinated biphenyls (PCBs), dioxins, and other toxic chemicals increase the toxicities of the individual compounds. This combination is more dangerous to ecosystems and has the potential to harm human health by accumulating in the food chain. The endocrine-disrupting effects of nitro-PAHs underline the necessity of decreasing exposure to these compounds and ensuring that they are effectively controlled to minimize the danger to human health and the environment.

5.5. *Persistence of nitro-PAHs*

The persistence of nitro-PAHs in the environment is another significant ecological concern. Because of their above mention physico-chemical nature, these compounds display exceptional resistance to natural degradation processes once introduced, potentially extending their shelf life for years to decades. Nitro-PAHs have been found in distant places such as the Antarctic, highlighting their pervasive and enduring character beyond discrete emission sources (Minero et al., 2010). The diverse effects of nitro-PAHs persistence have significant implications for the delicate ecological balance within impacted areas (Vasiljevic et al., 2021). Moreover, the persistent nature of nitro-PAHs exacerbates the aforementioned ecological impacts. The key problem is the prolonged exposure these substances impose on organisms, which might result in chronic toxicity. This prolonged presence raises concerns about the long-term health and sustainability of the species that live in these environments, which extends beyond the immediate ecological implications (Li et al., 2023). Due to the persistent nature of these pollutants, areas with previous contamination or continuing nitro-PAH sources face increased hazards.

5.6. *Bioaccumulation*

In terms of environmental effects, the bioaccumulation of nitro-PAHs appears as a crucial concern, exacerbating their ecological implications (Huang et al., 2014). This phenomenon involves a notable increase in these substances can grow several-fold from one trophic level to the next as they ascend the food chain (Tarazona, 2024; Zong et al., 2023). These chemicals can be absorbed by primary producers, such as plants or algae, from polluted soils or water. The nitro-PAHs are subsequently passed on to higher trophic levels by herbivores who consume the contaminated plants (Patel et al., 2020). Predatory animals at the top of the food chain, such as colossal fish or birds of prey, can accumulate substantial nitro-PAHs in their tissues. The consequences of bioaccumulation extend beyond individuals and affect entire ecosystems. This process has the potential to alter community structure, reduce biodiversity, and negatively impact overall ecosystem performance. Bioaccumulated nitro-PAHs can have negative results such as decreased productivity, nitrogen cycle disruption, and habitat destruction in aquatic environments, where the impacts are most evident.

5.7. Nitro-PAHs metabolism in organisms

Nitro-PAHs were rapidly metabolized via a variety of delivery routes, notably ring oxidation, and nitroreduction, followed by conjugation and excretion, mostly via feces and urine (Huang et al., 2023; Toriba et al., 2007). Their varied metabolic processes are controlled by administration routes and include gut microbiota, which aids nitroreduction and deconjugation and may potentially improve enterohepatic circulation (Claus et al., 2016; Sousa et al., 2008). These mechanisms include nitroreduction, nitroreduction with esterification (similar to acetylation), ring oxidation, and combinations of these, with bacteria preferring nitroreduction and fungi preferring ring oxidation (Mou et al., 2023). Cytochrome P450 enzymes play a critical role, with isomers and species differences impacting kinetics and routes. Nitroreduction of nitro-PAHs includes several enzymatic processes, including one and/or two-electron transfers, with bacterial activity playing a significant role in in-vivo nitroreduction in the intestinal tract (Ask et al., 2004). In nitroreduction, mammalian cells use a variety of enzymes (DT-diaphorase, xanthine oxidase, and cytosolic aldehyde oxidase), whereas oxidative metabolism, especially in Phase I, relies on Cytochrome P450 enzymes (Zhou et al., 2022). Recent research suggested that oxidative metabolism, such as 2-nitronaphthalene in human lymphoblastoid cells, has a role in genotoxicity (Clerg´e et al., 2019). Phase I, Phase II, and Phase III are the three major stages of the oxidative metabolism process. Cytochrome P450 enzymes, a subfamily of heme-containing monooxygenases, play a critical role in phase I. The insertion of a hydroxyl group into the nitro-PAHs molecule is catalyzed by these enzymes, resulting in the synthesis of hydroxylated nitro-PAHs (Li et al., 2023; Mokkawes et al., 2023). The nitro group is frequently reduced to an amino group during this hydroxylation event. Depending on the precise chemical structure of the nitro-PAHs, the ensuing hydroxylated or aminated metabolites may undergo further Phase I processes such as oxidation, reduction, or epoxidation. These processes try to make the metabolites more water soluble, allowing for easier removal. Phase II metabolism comprises conjugation processes that increase the water solubility of the metabolites, making them more excretable (Pegram and Chou, 1989; Zhang et al., 2023). Conjugation reactions usually include the addition of a polar group to Phase I metabolites. Glucuronidation, sulfation, and glutathione conjugation are all typical Phase II processes (Peng et al., 2023). Glucuronic acid is linked to the hydroxyl group of Phase I metabolites by glucuronosyltransferase enzymes in glucuronidation. Sulfation is the addition of a sulfate group to a metabolite catalyzed by sulfotransferase enzymes (Kaci et al., 2023). The attachment of a glutathione molecule to the metabolite is the consequence of glutathione conjugation, which is performed by glutathione S-transferases. These conjugated versions are more water-soluble and less poisonous, making them easier to excrete. The transport and excretion of conjugated metabolites from cells is part of Phase III metabolism (Phang-Lyn and Llerena, 2023). Efflux transporters, such as ATP-binding cassette (ABC) transporters, are essential in removing conjugated metabolites from cells, notably in the liver and kidneys (Kroll et al., 2021). Once in circulation, these metabolites are delivered to the kidneys for urine elimination or to the bile for feces excretion. The role of oxidative metabolism in mammalian systems may be more important. Nitro-PAHs have different mutagenic activation mechanisms. Some are activated by deacetylation or O-acetylation, resulting in electrophilic molecules that bind to DNA, but others, such as 6-nitrobenzo[*a*]pyrene, need oxidation to produce mutagenesis, similar to benzo[*a*]-pyrene (Arlt, 2004; Yun et al., 2020). This variety highlights the many and complicated processes by which nitro-PAHs produce mutagenesis effects.

6. Microbial remediation of nitro-PAHs

Microorganisms, including fungi and bacteria, can break down nitro-PAHs using a sequence of complex enzymatic processes. These processes take place in a variety of environmental circumstances, including both oxygen-rich (aerobic) and oxygen-depleted (anaerobic) environments (Chen et al., 2023; Salari et al., 2022). The aforementioned processes provide an all-encompassing microbial remediation mechanism, principally based on discrete stages: enzymatic reduction of nitro groups, ring cleavage, formation of central intermediates, and mineralization and assimilation (Li et al., 2023; Pandolfo et al., 2023). The microbial enzymatic reduction of the nitro groups is the first step in the aerobic biodegradation of nitro-PAHs (Claus, 2014). This enzymatic process transfers the electrons to the nitro group causing a chemical transformation, breaking the nitrogen-oxygen bond and converting the nitro group into an amino group (NH_2) (Penning et al., 2022). This reduction alters the chemical structure of the nitro-PAHs, lowering its overall complexity and making it more susceptible to future microbial enzyme breakdown processes. Nitroreductases are essential enzymes in this reduction process. Nitroreductases are frequently selective to nitro-PAHs and have variable substrate specificities. After this process, the aromatic ring of nitro-PAHs is hydroxylated which improves the water solubility of amino-containing nitro-PAHs, allowing them to dissolve in water (Baboshin and Golovleva, 2012). This enzymatic mechanism integrates hydroxyl groups onto the aromatic ring structure, which is aided by dioxygenases [Rieske non-heme ring-hydroxylating oxygenase (RHO)] or cytochrome P450 monooxygenases (CYP450s) (Peng et al., 2008). These intermediates are subsequently rearomatized by dehydrogenases, restoring the ring's aromaticity. In the following stage, the dihydroxy aromatic intermediates serve as substrates for intradiol or extradiol dioxygenases. Extradiol dioxygenases break the aromatic ring between two hydroxyl groups in an ortho position, whereas intradiol dioxygenases cleave the ring in a meta position (Seo et al., 2009). This cleavage is mediated by oxygen, resulting in the generation of intermediates such as catechol. Following dioxygenase cleavage, catechol is converted into a primary alcohol, which is then oxidized to generate the equivalent aldehyde (Das et al., 2023). This aldehyde is subsequently converted into fatty acids, triggering a series of biochemical events including beta-oxidation that results in acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle, creating CO_2, H_2O, ATP, and NADH (Das et al., 2023; Peixoto et al., 2011) (Fig. 2).

Anaerobic biodegradation, in contrast to aerobic biodegradation, often takes place when nitrate (NO^{3-}), sulfate (SO_4^{2-}), manganese (IV), iron (Fe^{3+}), carbon dioxide (CO_2), or methanogens are present. The reductive process is an effective mechanism in anaerobic nitro-PAHs degradation, in which nitro groups are enzymatically reduced to amino groups (Chen et al., 2023; Dhar et al., 2019). Nitroreductases, enzymes synthesized by particular anaerobic bacteria, often catalyze this reduction (Wang and Tam, 2019). Nitroreductases transport electrons from electron donors to the nitro group, causing it to be reduced and leading to the generation of amino-PAHs or hydroxylamine intermediates. Following the reduction stage, anaerobic microbes breakdown the amino-PAHs via several metabolic pathways. One standard process is the aromatic ring reduction route, which produces cyclohexadiene and cyclohexenes by sequentially reducing the aromatic rings of nitro-PAHs (Zhang et al., 2022). Various enzymes, such as reductive dehalogenases, catalyze this reduction, which removes halogen substituents from the aromatic rings. The resultant cyclohexadiene and cyclohexenes can be metabolized through different enzymatic processes to create more specific aromatic or aliphatic molecules (Ang et al., 2018). In addition to the aromatic ring reduction process, anaerobic microbes may use alternative mechanisms to degrade nitro-PAHs. Hydroxylation introduces hydroxyl groups into aromatic rings, whereas the

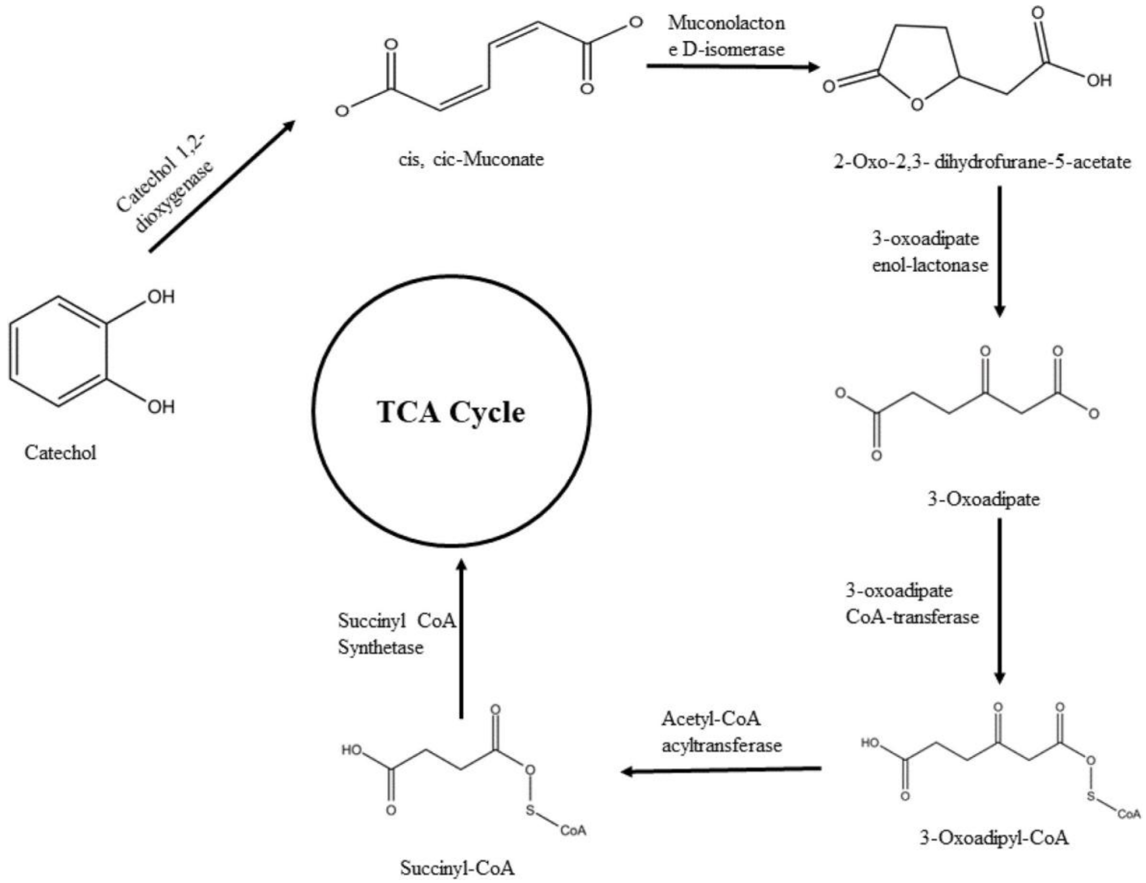


Fig. 2. The enzymatic pathway for Catechol degradation.

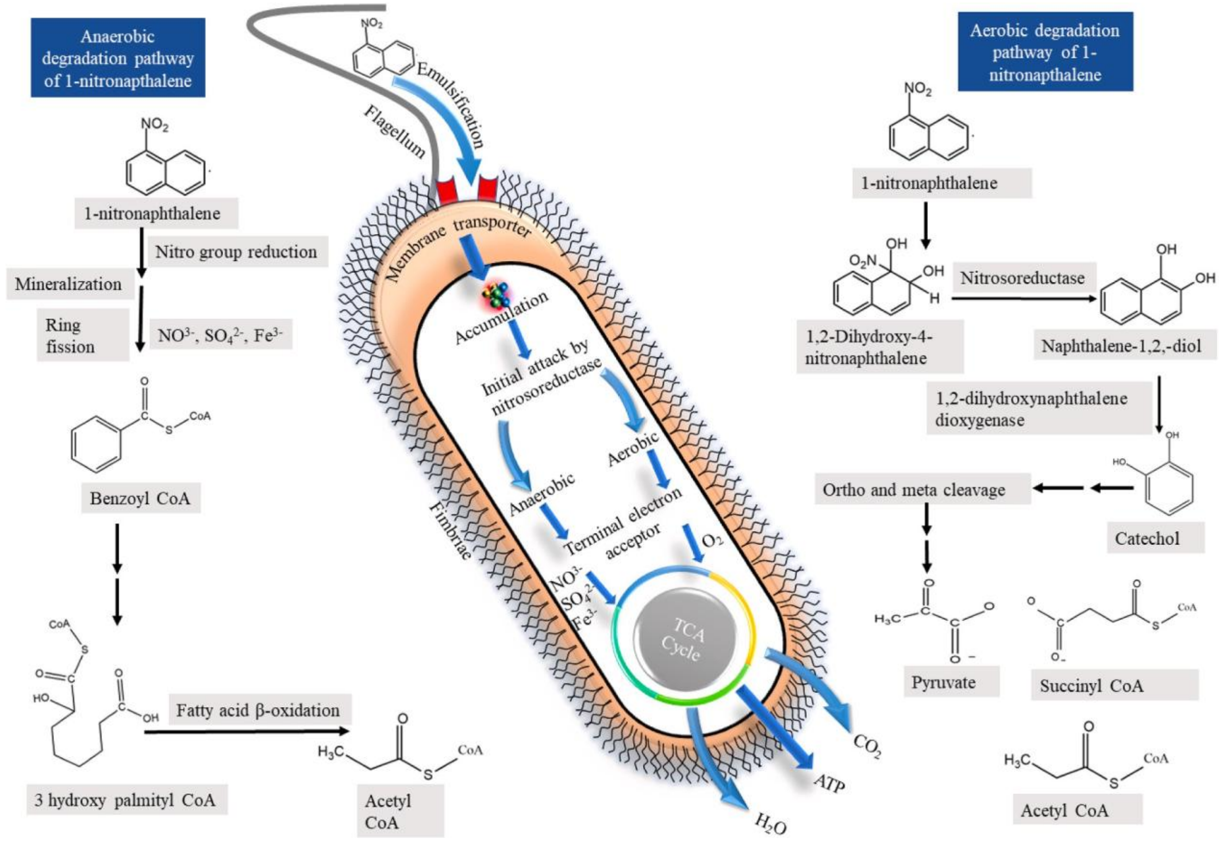


Fig. 3. Microremediation pathways of nitro-PAHs.

methyl group oxidation pathway oxidizes and removes methyl groups from the nitro-PAH structure (Rayaroth et al., 2023). These routes involve various enzymes, including hydroxylases and oxidoreductases, promoting the processes necessary for the breakdown of nitro-PAHs. The overall microremediation pathways are illustrated in Fig. 3.

6.1. *Bacterial remediation of nitro-PAHs*

With their extraordinary flexibility and metabolic plasticity, bacteria have evolved over billions of years to acquire energy from a diverse spectrum of chemicals effectively. They have evolved into nature's most excellent scavengers, capable of surviving on a wide range of chemicals. Bacteria's capacity has made them essential in environmental remediation, where they are utilized to decompose and eliminate numerous hazardous compounds (Ghosal et al., 2016). Microbes belonging to the genera *Pseudomonas*, *Sphingomonas*, *Micrococcus*, *Bacillus*, *Mycobacterium*, *Acinetobacter*, and *Xanthomonas* have been widely examined and recorded for their ability to degrade aromatic hydrocarbons. Bacteria prefer oxygen-rich environments for nitro-PAH metabolism, and converting these molecules into more straightforward and less dangerous chemicals requires a series of enzyme processes. One important group of bacteria involved in nitro-PAHs degradation is the genus *Mycobacterium*. These bacteria are well-known for their ability to use a variety of organic molecules as carbon sources. Nitroreductases are enzymes found in *Mycobacterium* species that may reduce the nitro group present in nitro-PAHs, hence commencing the breakdown process (Manina et al., 2010). Bandowe and Meusel (2017) emphasized the metabolic flexibility of *Mycobacterium* sp., a bacterial species capable of metabolizing nitro-PAHs via both oxidative and reductive routes. When subjected to aerobic conditions and oxygen, 1-nitropyrene oxidizes, yielding 1-nitropyrene-9,10-dihydrodiol and 1-nitropyrene-4,5-dihydrodiol. Without oxygen, 1-nitropyrene is reduced, forming 1-aminopyrene (Bandowe and Meusel, 2017). Furthermore, Li et al.'s findings from 2023 showed that the *Sphingobium* sp. strain JS3065 transformed 1-nitronaphthalene into 1,2-dihydroxynaphthalene, a precursor in the route for naphthalene degradation (Li et al., 2023). This finding demonstrated the tremendous potential of using microbes to increase the number of substrates suitable for efficient remediation, particularly for persistent nitroaromatic compounds. Some common microorganisms are effective at reducing nitro-PAHs. It includes *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Sphingomonas paucimobilis*, *Burkholderia cepacian*, *Ralstonia pickettii*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia* (Pothuluri, 1996; Yan and Wu, 2017).

6.2. *Fungal remediation of nitro-PAHs*

Fungal species degrade organic pollutants well due to various favorable properties (Panigrahy et al., 2022). For starters, fungi are well-known for their resistance to high concentrations of refractory chemicals (Harms et al., 2011). Their systems allow them to resist and metabolize these complex and long-lasting contaminants. Fungi may also flourish in harsh environments like high temperatures and low pH levels. This versatility allows them to colonize and break down nitro-PAHs when other species struggle (Deshmukh et al., 2016). Although most fungi are unable to use nitro-PAHs as primary carbon and energy sources, they may co-metabolize nitro-PAHs, resulting in the generation of a variety of oxidized by-products including, on occasionally, carbon dioxide (CO_2).

Extracellular enzymes produced by fungi include ligninolytic enzymes (including lignin peroxidases, manganese peroxidases, and laccases), cytochrome P450 monooxygenases, and dioxygenases, which are involved in the breakdown of nitro-PAHs (Baker et al., 2019). These enzymes allow fungi to commence the breakdown of complicated aromatic structures and enhance subsequent metabolic activities (Kumar and Chandra, 2020). The white-rot fungi, which include species from the genera *Phanerochaete*, *Trametes*, and *Pleurotus* are one well-studied group of fungi with sophisticated aromatic degrading abilities. These fungi have ligninolytic enzymes that aid in the breakdown of a range of organic contaminants, including nitro-PAHs. They can effectively break down the aromatic rings of nitro-PAHs, forming smaller, more easily degradable molecules (Arora, 2019). The black-rot fungus, particularly from the genus *Aspergillus* is another group of fungi recognized for their capacity to breakdown aromatic hydrocarbons. Extracellular enzymes, such as cytochrome P450 monooxygenases are produced by these fungi allowing them to breakdown and metabolize aromatic hydrocarbons (Alegbeleye et al., 2017). In addition to white-rot and black-rot fungi, several additional fungal species, including representatives of the genera *Penicillium*, *Fusarium*, and *Bjerkandera* have been found as effective aromatic hydrocarbon degraders (Ghosal et al., 2016). These fungi have unique enzyme systems that break aromatic hydrocarbon and utilize the resultant metabolites as carbon and energy sources.

6.3. *Genes responsible for degradation*

Several investigations have discovered numerous clusters of highly conserved nitro-PAH-catabolic genes in several bacterial species engaged in nitro-PAHs degradation (Huang et al., 2023; Seo et al., 2009)). The nitroreductase gene family is a well-known collection of genes involved in nitro-PAH degradation (Caballero et al., 2005). These genes code for nitroreductase enzymes, which catalyze the first reduction of the nitro groups found in nitro-PAH. Nitroreductases are essential in the enzymatic conversion of nitro-PAHs into amino-PAHs or hydroxylamine intermediates, which serve as critical intermediates for subsequent degradation stages. Specific catabolic genes, such as nar, phd, nid, and pdo, are widely present in gram-positive bacterial species such as *Rhodococcus*, *Mycobacterium*, and *Nocardioides*, which are recognized for their capacity to digest aromatic hydrocarbons (Bengtsson et al., 2013). Catabolic genes such as nah, nag, ndo, pah, and phn, on the other hand, are widely found in Gram-negative bacterial species such as *Pseudomonas*, *Ralstonia*, *Burkholderia*, *Sphingomonas*, and *Polaromonas*, which also have aromatic hydrocarbons degradation capabilities (Yagi and Madsen, 2009; Zhou et al., 2001). One of the most widely conserved nitro-PAH-catabolic genes is nag, present in *Sphingobium* sp. and *Ralstonia* sp (Jones et al., 2003; Li et al., 2023). This gene contributes to the degradation of 1-nitronaphthalene to 1,2-dihydroxynaphthalene. nahAc, present in various gram-negative bacterial species, is another highly conserved nitro-PAH-catabolic gene (Ma et al., 2006). The existence of this gene in a variety of microbial species can be linked to environmental horizontal gene transfer, a process in which genetic material is shared between various organisms in the environment (Goyal, 2022). Horizontal gene transfer has played an essential role in spreading nitro-PAHs degradation capabilities throughout bacterial populations, allowing numerous bacterial species to acquire nitro-PAHs-catabolic genes. The distinct clusters of highly conserved nitro-PAH-catabolic genes in different bacteria demonstrate microorganisms' genetic variety and flexibility in PAH degradation. These genes are critical in the metabolism of nitro-PAH molecules. Understanding the genetics of nitro-PAHs degradation gives vital insights into the microbial remediation potential and opens up chances for designing successful bioremediation solutions.

6.4. *Regulatory factors*

The efficacy of nitro-PAHs microbial remediation can be regulated by various variables that affect enzyme activity and gene expression within the microbial population. These characteristics can impact the rate and efficiency of degradation as well as the persistence of nitro-PAHs in the environment. Temperature, pH, and food availability all influence the microbial breakdown of nitro-PAHs (Bhattacharjee et al., 2022; Duran and Cravo-Laureau, 2016). Microbes responsible for nitro-PAHs degradation are generally acclimated to certain environmental circumstances, and changes in these settings can significantly influence their capacity to break down the compounds (Mohapatra and Phale, 2021). For example, high temperatures can hasten deterioration, whereas low temperatures can slow it down. On the other hand, the pH of the environment might alter degradation since many microbial strains are acclimated to specific pH ranges and cannot operate well outside of these ranges (Muskus et al., 2022). The makeup of the microbial population in the environment can also impact the degradation of nitro-PAHs (Fernández-Luqueño et al., 2011). The consortium's many microbes can breakdown various pollutants. Furthermore, the employment of microbial consortia might boost the robustness of the degradation process since the presence of several microorganisms can offer redundancy and raise the possibility of effective degradation even in the face of unfavorable circumstances (Zhang and Zhang, 2022). The genetics of the microbial strains participating in nitro-PAHs degradation can also impact degradation rate and efficiency. Different microbial strains degrade different kinds of nitro-PAHs in different ways, and their effectiveness can be modified by variables such as their metabolic pathways and the presence of certain enzymes (Rafeeq et al., 2023). The presence of strains with improved metabolic pathways or enhanced enzymes can increase the rate and efficiency of degradation (Yaashikaa et al., 2022). Other environmental compounds can hinder nitro-PAHs microbial breakdown (Pandolfo et al., 2023). These inhibitors can disrupt microbial metabolic processes or the enzymes responsible for chemical breakdown. Heavy metals, surfactants, and other contaminants are frequent inhibitors. The presence of these inhibitors can limit the pace and efficiency of degradation and, in extreme situations, even render nitro-PAHs degradation impossible. Substrate availability is critical in determining nitro-PAHs microbial degradation since it directly influences the capacity of microbial communities to utilize and degrade these chemicals. Substrate availability is the term used to describe the accessibility and concentration of substrates, such as nitro-PAHs, in the environment. It depends on several elements, including concentration, dispersion, interactions with microbial communities, and persistence (Shi et al., 2021; Wang et al., 2022). Microorganisms require a sufficient concentration of substrates, such as nitro-PAHs, to maintain their development and metabolic activity (Okpokwasili and Nweke, 2006). Higher substrate concentrations give a higher carbon supply for microbial populations, enabling increasing microbial development and boosting their capability for destruction (Wang et al., 2022). However, extremely high substrate availability might inhibit microbial activity, exceeding the microbial community's tolerance threshold. Strategies to improve microbial remediation of nitro-PAH-contaminated settings may be designed with the aid of manipulating substrate availability. By optimizing substrate accessibility, innovative methods can be developed to treat nitro-PAHs contamination and boost environmental cleaning effectively.

7. Phytoremediation of nitro-PAHs

Phytoremediation is an eco-friendly, efficient biological strategy for tackling lower levels of pollutants present in soil or water (Sarma et al., 2019; Zulkernain et al., 2023). The mechanism behind phytoremediation incorporates several activities that take advantage of plants' and related microbes' particular capacities to degrade, stabilize, or remove these pollutants from the environment (Mocek-Płóciniak et al., 2023; Tang, 2023). Plant absorption and metabolism are one of the primary processes involved in nitro-PAHs phytoremediation. Nitro-PAHs can be taken up by some plant species via their roots from the soil or water; later, they can be metabolized and converted into less harmful or more readily degradable chemicals once within the plant. Enzymatic reactions in the plant, such as oxidation, reduction, and conjugation, can alter the chemical structure of nitro-PAHs (Hernández-Vega et al., 2017). Enzymes found in plant tissues, such as cytochrome P450 monooxygenases and glutathione S-transferases, are frequently responsible for these metabolic processes (Cheng et al., 2022).

Rhizodegradation is another significant pathway in nitro-PAHs' phytoremediation (Hussain et al., 2018). The rhizosphere, or the soil zone around plant roots, is critical to this process. Plants release root exudates (a variety of organic chemicals into the rhizosphere, referred to as root exudates) that can provide nutrients and energy to soil microbes (Gogoi and Sarma, 2023; Singha and Pandey, 2021). Some of these microbes include *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Sphingomonas paucimobilis*, *Ralstonia pickettii*, *Stenotrophomonas maltophilia*, *Cunninghamella elegans*, and *Aspergillus niger* can break down nitro-PAHs via enzymatic processes (Pérez-Pantoja et al., 2019). The interaction between plants and these rhizosphere bacteria can improve nitro-PAHs breakdown and boost their biodegradation, possessing the capability to degrade nitro-PAHs through enzymatic reactions.

A versatile and environmentally friendly strategy for cleaning up nitro-PAH-contaminated areas might be achieved via rhizodegradation, phytovolatilization, and phytostabilization processes acting in concert (Barroso et al., 2023). While several grasses, legumes, aquatic weeds, and genetically engineered plants have been shown to break down primary PAHs, their capacity to reduce nitro-PAHs has not been widely examined (Xie et al., 2012). Further research and development are required to optimize and implement phytoremediation procedures properly, considering site-specific circumstances and selecting appropriate plant species for optimal remediation outcomes.

8. Biostimulants assisted remediation of nitro-PAHs

Biostimulants-assisted nitro-PAH remediation is emerging as a promising and sustainable alternative to conventional physical and chemical remediation approaches. Biostimulants are biologically derived compounds that can provide the nutrients and conditions needed to enhance the development and activity of microorganisms and plants involved in environmental remediation (Mandal et al., 2023). These substances can include vitamins, minerals, and organic compounds, and it has been proposed that the usage of these organic compounds increases the rate of breakdown and biotransformation of hydrocarbon contaminants in polluted environments (Etesami et al., 2023; Prasad et al., 2019). Biostimulants can also improve the plant's ability to detoxify the contaminants by activating its metabolic processes (Bartucca et al., 2022). Additionally, biostimulants can stimulate the production of enzymes that degrade nitro-PAHs into less toxic compounds, which can help to reduce nitro-PAHs toxicity and improve the plant's overall health and performance (Yakhin et al., 2017). In addition to direct benefits, biostimulants can indirectly affect nitro-PAHs cleanup. For example, they can encourage plant development, which can assist in limiting human and wildlife exposure to nitro-PAHs by minimizing soil erosion and boosting the growth of vegetation, which can function as a physical barrier to contamination. Several sorts of biostimulants have been proposed for their ability to remediate nitro-PAHs, which are listed below.

8.1. *Plant growth promoting rhizobacteria (PGPR)*

PGPR has gained much interest among biostimulants because of its ability to remediate nitro-PAHs (Hoang et al., 2021). PGPR are soil-dwelling bacteria that create symbiotic connections with plant roots. They have been discovered to have various favorable benefits on plant growth, including enhanced nutrient absorption, greater resilience to stress, and higher growth and yield (Maleki et al., 2023). Some rhizobacterial strains have also been reported to be capable of bioremediating nitro-PAHs, making them attractive candidates for environmental bioremediation (Tiwari et al., 2017). One of the primary ways PGPR can remediate nitro-PAHs is the synthesis of enzymes such as laccases and peroxidases, which are involved in the breakdown of these compounds. For example, *Pseudomonas mendocina* (Wang et al., 2021), *Pseudomonas stutzeri* (Singh and Tiwary, 2017), *Pseudomonas aeruginosa* (Yanhua et al., 2023), *Pseudomonas putida* (García-Franco et al., 2023), *Rhizobium leguminosarum* (Hemati et al., 2023), *Rhizobium etli* (Gul et al., 2023), *Rhizobium meliloti* (Liu et al., 2022), *Bacillus cereus* (Valizadeh et al., 2023), *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus megaterium* (Kirthi et al., 2023), *Ochrobactrum anthropic*, *Ochrobactrum intermedium*, *Ochrobactrum tritici*, *Ochrobactrum pseudogrignonense* (Navarro-Torre et al., 2023), *Streptomyces albus*, *Streptomyces viridosporus*, *Streptomyces avermitilis*, and *Streptomyces griseus* (Salwan et al., 2022), are some PGPR strains have been found to produce laccases and peroxidases, which are involved in the degradation of nitro-PAHs. Moreover, PGPR has been shown to boost the population of other microorganisms engaged in nitro-PAHs bioremediation, such as fungi and other bacteria (Brown et al., 2022).

8.2. Mycorrhizal fungi

Mycorrhizal fungi are another popular biostimulants. This diverse collection of fungi forms mutualistic interactions with plant roots and has sparked interest in recent decades for their potential use as biostimulants in the regeneration of contaminated soils (Bartucca et al., 2022; Hijri, 2023). These fungi are well-known for their capacity to improve plant development and health by offering access to nutrients and water in the soil (Wang et al., 2023). They have been discovered to have an essential role in the bioremediation of hydrocarbons via numerous methods, including promoting microbial populations engaged in the biodegradation of pollutants (Ingrid et al., 2016). Furthermore, mycorrhizal fungi have been shown to produce enzymes such as laccases and peroxidases, which have been implicated in nitro-PAHs' biodegradation. The mycorrhizal fungus has been demonstrated to boost the pace of biodegradation and the total amount of hydrocarbon breakdown. These findings show that mycorrhizal fungi might be a helpful tool in the bioremediation of polluted soils in the future, particularly those contaminated with nitro-PAHs. Several species of mycorrhizal fungi are effective at reducing hydrocarbons, including *Glomus mosseae*, *Glomus intraradices*, *Glomus aggregatum*, *Gigaspora margarita*, *Rhizophagus irregularis*, *Funneliformis mosseae*, *Laccaria bicolor*, *Paxillus involutus*, *Suillus bovinus*, *Scleroderma citrinum*, and *Hebeloma cylindrosporum* (Arora, 2019).

8.3. Seaweed extracts

Seaweed extracts are another potential biostimulants that have attracted attention as a possible bioremediation due to the presence of chemicals such as enzymes, amino acids, and polysaccharides that might boost the development and activity of microorganisms involved in the biodegradation of nitro-PAHs (Top et al., 2023; Znad et al., 2022). Numerous studies have shown promising results in utilizing seaweed extracts for hydrocarbon cleanup. One such study investigated the efficacy of using the brown seaweed *Ascophyllum nodosum* extract in degrading PAHs in contaminated soil, significantly reducing the concentration of these pollutants (Boudh et al., 2019). Similarly, another study highlighted the potential of the green seaweed *Ulva lactuca* extract to break down PAHs in polluted water, thereby reducing their toxicity (Areco et al., 2021). These findings underscore the potential of seaweed extracts as a valuable tool for mitigating hydrocarbon pollution.

8.4. Compost

Compost is another biostimulants widely used to treat contaminated soil (Sayara and Sánchez, 2020; Visconti et al., 2023). Compost is formed from decomposing organic materials, such as plant waste, food scraps, and manure (Qian et al., 2023). Compost is rich in organic matter, and nutrients, when it is added to polluted soil it helps the growth and development of microorganisms and plants. Due to this characteristic of compost they indirectly helps the nitro-PAHs' degrading process (Visconti et al., 2022).

Using biostimulants to help with nitro-PAHs cleanup has various advantages, including environmental friendliness, cost-effectiveness,

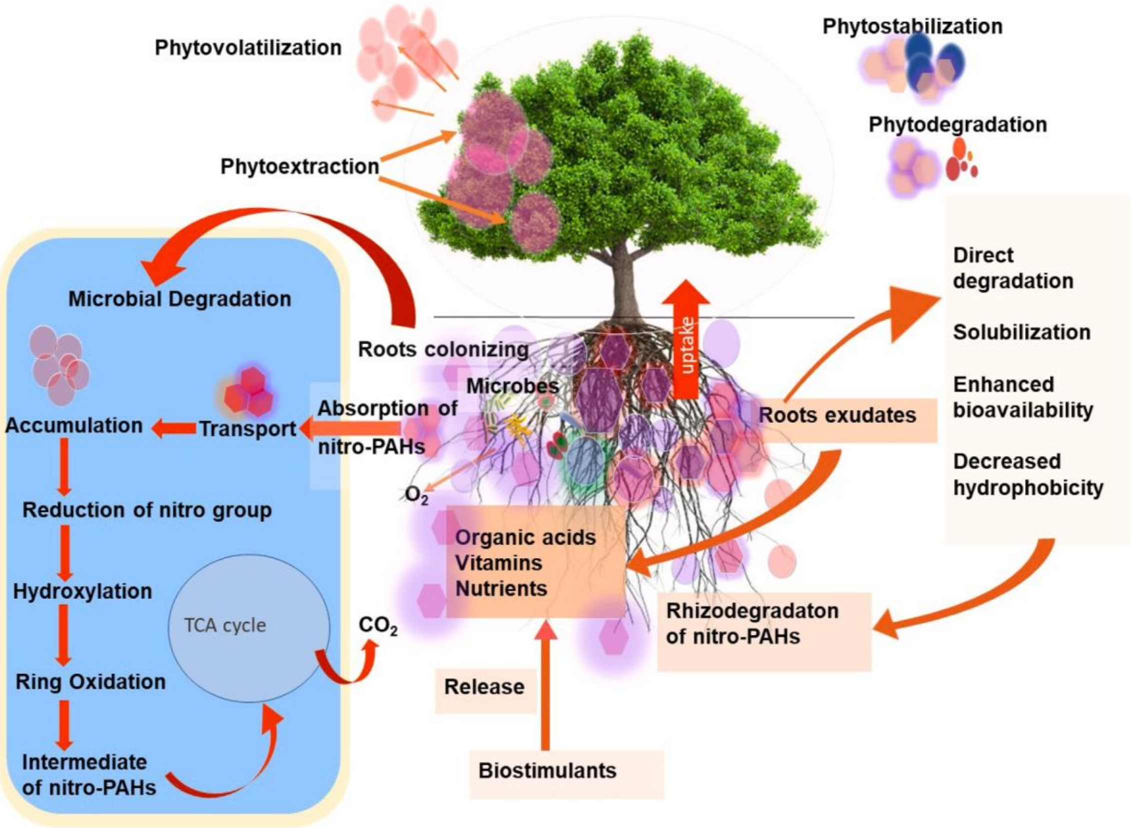


Fig. 4. Synergistic approach for microbes and biostimulants assisted phytoremediation of nitro-PAHs.

and increased microbial activity and plant health (Kumari et al., 2022). However, it has certain drawbacks, including fluctuating efficacy, limited understanding, a lack of standardization, complexity, and limited durability. Despite these restrictions, biostimulants may help remove nitro-PAHs from polluted environments.

9. Biostimulants and microbes assisted phytoremediation

There are considerable downsides to phytoremediation. Compared to typical treatments, it is a slow process that needs long-term monitoring and supervision (Kristanti and Hadibarata, 2023). Phytoremediation efficacy may be limited by factors such as the availability of suitable plant species, the accessibility of toxins, and environmental conditions. Furthermore, it may not be suitable for highly contaminated areas or time-sensitive remediation requirements (Aghili and Golzary, 2023; Kafle et al., 2022). Considering these disadvantages, combining biostimulants and microorganisms can become feasible for nitro-PAHs phytoremediation. The biostimulants and microbes-assisted phytoremediation of nitro-PAHs comprises a mix of biological and chemical processes (Fig. 4).

The absorption of nitro-PAHs by plant roots is hypothesized to be the initial stage in this process. The root surface area is a crucial factor in the plant's ability to absorb contaminants, and biostimulants can be used to increase the root surface area and promote plant growth (Szopa et al., 2023). Increasing the root surface area allows the plant to absorb and degrade nitro-PAHs more remarkably. When nitro-PAHs are absorbed by the plant, they are transferred to the plant's leaves and stems, where they can be annihilated through the phytoextraction and phytovolatilization processes (Sitarska et al., 2023). Enzymes are necessary for the plant to breakdown the nitro-PAHs. Microorganisms that colonize plant roots, such as rhizosphere microorganisms and mycorrhiza, can offer these enzymes (Begum et al., 2023; Singh et al., 2023). These microbes can produce enzymes that break down nitro-PAHs and boost the plant's ability to absorb and digest pollutants by providing more nutrients and enzymes. The plant and its accompanying microbes can degrade nitro-PAHs, producing intermediate metabolites, some of which are poisonous or persistent. To properly remediate polluted soils and water bodies, it is critical to monitor the degradation of nitro-PAHs and the creation of intermediate metabolites, as well as to take adequate precautions to limit their potential impact on human health and the environment. In addition to degrading nitro-PAHs, biostimulants can enhance plant stress tolerance, which is especially important for plants living in polluted settings. Biostimulants can assist assure the long-term viability of the phytoremediation process by enhancing plant stress tolerance. Phytoremediation has the potential to be an effective, sustainable, and cost-efficient solution to polluted site cleanup by combining the use of biostimulants and microbes.

10. Conclusion and future perspective

Nitro-PAHs have a substantial effect on soil fertility, microbial populations, and bio-diversity. And also affects on human health by inducing inflammation, mutagenesis, and carcinogenesis. Current toxicity data, on the other hand, frequently concentrates on individual substances at exaggerated doses, ignoring genuine environmental exposure consequences. Although the structural similarities between nitro-PAH derivatives and parent chemicals imply comparable effects on the human body, research on their health effect is scarce. Understanding the existence, bioavailability, and toxicity of nitro-PAH derivatives in environmental matrices is critical given their potential for toxicity and mutagenicity. The scarcity of studies emphasizes the importance of doing rigorous research to appropriately determine their impacts. Microbes and biostimulants-assisted remediation technologies hold promise for future cleaning up nitro-PAHs contaminated regions. However, several challenges need to be overcome for successful implementation. The diverse nature of nitro-PAHs requires specialized microbial strains with sufficient enzyme capabilities for efficient degradation. Identifying and characterizing these strains is complex due to the diverse microbial populations and interactions in polluted settings. Further research is needed to investigate microbial diversity and activity to aid in the discovery of new degraders and their functional genes. Co-contaminants like heavy metals and organic pollutants hinder nitro-PAHs breakdown by interfering with microbial activity, so developing methods to reduce their effects and boost microbial resistance is essential. Optimizing environmental conditions such as temperature, pH, and nutrient availability is crucial for sustaining microbial growth and activity levels. Selecting appropriate biostimulants is challenging, considering the different nutrients, carbon sources, electron acceptors, and donors that stimulate specific metabolic pathways and microbial populations. Determining optimal biostimulant application rates is crucial to avoid hindering growth or causing unintended environmental issues. Monitoring and risk assessment are necessary to understand the environmental impact of biostimulant residues. Advanced monitoring techniques can evaluate microbial activity and degradation rates in real time. Improved biostimulant formulations and delivery technologies, such as nanoparticles and biochar, can enhance the effectiveness and efficiency of cleanup. Long-term monitoring, compliance with environmental standards, and regulatory procedures are also required for successful clean-up. Increased research, government actions, and public awareness are required to fully appreciate the impacts of nitro-PAHs. Strict international coordination and strict monitoring by regulatory bodies are required to control nitro-PAHs manufacturing and argue for a ban on these pollutants. Monitoring contaminated areas at the same time is critical for reducing their impact on ecosystems and human health.

CRediT authorship contribution statement

Hemen Sarma: Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Writing – review & editing. **Bhoirob Gogoi:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft. **Chung-Yu Guan:** Writing – review & editing. **Chang-Ping Yu:** Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used [https://app.grammarly.com//(License version), Servier Medical ART: SMART] in order to improve the readability and preparation of figures. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix III

SEMINARS/WORKSHOPS ATTENDED

1. International Seminar on Interdisciplinary Approaches For Sustainable World (ISF-25) organized by Chandra Nath Bezbaruah College, Bokakhat in collaboration with Bodoland University, Kokrajhar, Assam (2025).
2. ICSSR Sponsored National Seminar on Environmental, Sustainable Development & Gender in North East India: Issues and Challenges organized by Kakojan College, Jorhat, Assam (2024).

IIS-25

INTERNATIONAL SEMINAR

On

"Interdisciplinary Approaches For a Sustainable World"

6th & 7th January, 2025

Certificate of Appreciation

This certificate is proudly presented to

Bhoirob Gogoi *of* **Bodoland University**

for excellence in Paper presentation (1st Prize) in the International Seminar on

"Interdisciplinary Approaches For a Sustainable World"

held on 6th & 7th January, 2025 at CNB College, Bokakhat, Assam, India,

Organised By CNB College, Bokakhat, Assam In collaboration with Bodoland University, Kokrajhar, Assam.

The title of his paper is " Synergistic Degradation of Nitro-PAHs by Co-Inoculum of Bacillus Strains: Implications for Eco-Friendly and Sustainable Remediation.

Dr. Surajit Dutta
Chief Patron

Dr. Hari Prasad Nath
Patron

Dr. Madhulika Dutta
Convenor

Mr. Debajit Saikia
Organizing Secretary

Organizing Committee, International Seminar, Bokakhat-785612, Assam, India

ICSSR

ICSSR Sponsored National Seminar

on

Environment, Sustainable Development & Gender in North East India: Issues and Challenges

Organized By:

Internal Quality Assurance Cell (IQAC), Kakojan College

In collaboration with

The Assam Kaziranga University, Jorhat

Estd. 1967

CERTIFICATE

This is to Certify that

~~Dr.~~/ Mr. /~~Mrs.~~/~~Ms.~~ Bhoirob Gogoi, Research Scholar *of* Bodoland University, Kokrajhar *has participated in the ICSSR Sponsored National Seminar held on 14th-15th March, 2024 at Kakojan College, Jorhat, Assam and presented a paper titled* Biostimulants Assisted Rhizoremediation of Nitro-PAHs in Crude Oil Contaminants Soil

(Dr. Rashmi Rekha Saikia) Principal & Chairperson National Seminar Organizing Committee	*(Dr. H R Kamath)* Registrar The Assam Kaziranga University Jorhat	*(Dhrubajyoti Saikia)* Coordinator IQAC Kakojan College	*(Dr. Sikhamoni Gogoi)* Convener National Seminar Organizing Committee