Chapter II: Review of Literature

2.1. Sources and Environmental Behavior of nitro-PAHs

Nitro-PAHs have been formed from a variety of sources, including natural and anthropogenic activities. The most common source of their production (directly or indirectly) is incomplete combustion of PAHs. Among the natural sources, lightning strikes produced nitro-PAHs. When lightning strikes, it generates high energy and heat, which can partially burn organic matter (such as plants, debris, or other carbon-based materials) in the environment. This process releases nitro-PAHs into the atmosphere. This incomplete combustion releases PAHs, which can undergo subsequent nitration processes to form nitro-PAHs (Lee, 2010). Volcanic eruptions are another natural source that initially releases PAHs originating from the combustion of organic material within volcanic rocks (Kozak et al., 2017; Remizovschi et al., 2020). The interaction of these volcanic PAHs with nitrogen oxides in the volcanic plume can lead to nitro-PAH formation (Mulder et al., 2019).

While natural sources like lightning strikes and volcanic eruptions contribute to the formation of nitro-PAHs through processes beyond human control, the anthropogenic causes, primarily stemming from the incomplete combustion of PAHs in industrial activities and vehicular emissions, pose a significant and preventable concern. Anthropogenic sources have a considerable impact on the quantity of nitro-PAHs in the environment as compared to their natural sources. Most of the nitro-PAHs originate either directly or indirectly from incomplete combustion processes that are triggered by humans. Also, nitro-PAHs vary from PAHs in that they are produced by incomplete combustion (coal, biomass, and automobile emissions) (Bandowe & Meusel, 2017). They can enter the environment with PAHs or form as a result of free radical (OH and NO_3) reactions, as well as homogeneous and heterogeneous processes (Cao et al., 2023; Eldos et al., 2022).

Prior reports emphasized diesel engines have an important influence on nitro-PAH emissions (Karavalakis et al., 2010; Paim et al., 2023). Karavalakis et al. (2011) reported that diesel engines released 7 nitro-PAHs specifically 1-nitronaphthalene, 2-nitronaphthalene, 2-nitrofluoranthene, 9-nitroanthracene, 1-nitropyrene, 6-nitrochrysene, and 6-nitroB[a]pyrene with concentrations ranging from 0.417 to 1.917 μg km^{-1}. Therefore, diesel engines are a significant source of nitro-PAH emissions, underscoring the need for stricter regulations to reduce their environmental impact. Diesel engines are a major source of nitro-PAHs, but alternative fuels like biodiesel have also been found to affect nitro-PAH emissions in different

ways. Biodiesel has emerged as another source of nitro-PAHs. Recently, Paim et al. (2023) revealed that 27 specific nitro-PAHs were formed during the combustion of biodiesel-ethanol blends. This finding complicates biodiesel's environment and raises questions about its sustainability (Paim et al., 2023). Contradictorily, Karavalakis, et al. (2011) revealed that the combustion of biodiesel might reduce some nitro-PAH concentrations in emissions, but it may potentially raise others (Karavalakis et al., 2011).

Similarly, ample amounts of nitro-PAHs, such as 1,8-dinitronaphthalene, 5-nitroacenaphthene, 1,5-dinitronaphthalene, and nitronaphthalenes have been synthesized artificially for use in the manufacturing of explosive, dye, and colorant (Bandowe & Meusel, 2017). Interestingly, various places in Europe and the United States have been recognized as having nitro-PAH pollution, particularly those areas where the explosive was extensively used, mostly during World Wars I and II (Ju & Parales, 2010).

Oil spills are a major source of nitro-PAHs because they release PAHs, which react with nitrogen oxides to form nitro-PAHs. These pollutants contaminate air, water, and soil, posing risks to human health and ecosystems (Vasiljevic et al., 2021). These spills are caused by various petroleum industry activities, including extraction, transportation, refining, and accidental leaks or pipeline ruptures. For example, Zhao et al. (2015) reported that the explosion of an oil pipeline released 13 potent nitro-PAHs namely, 1-nitropyrene, 2-nitrofluorene, 6-nitrobenz(a)pyrene, 1,8-dinitropyrene, 1,6-dinitropyrene, 1,3-dinitropyrene, 6-nitrochrysene, 7-nitrobenz(a)anthracene, 2-nitrofluoranthene, 3-nitrophenanthrene, 9-nitrophenanthrene, 9-nitroanthracene, and 1-nitronaphthalene (Zhao et al., 2015).

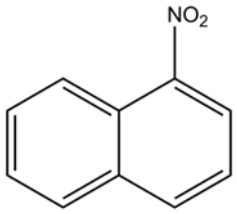
The nitro-PAHs occur in the atmosphere in two distinct phases: vapor and particulate (Zimmermann et al., 2013). For instance, 1-nitropyrene, 7-nitrobenz[a]anthracene, and 2-nitrofluoranthene are in the particulate phase, whereas 9-nitroanthracene exists in both the particulate and vapor phases (Gao et al., 2022; Lammel et al., 2020). On the other hand, 2-nitrofluoranthene, 2-nitronaphthalenes, and 2-nitropyrene are in the vapor phase (Wilson et al., 2020). These chemicals can be transported as vapors or by adhering to suspended particulate particles in the atmosphere. Finally, the atmospheric nitro-PAHs are accumulated in the soil matrix through both wet and dry deposition processes (Sarma et al., 2024). Due to their high sorption capacities, they attach tightly to solid soil particles. However, they can be transferred to deeper soil layers via colloidal-assisted transport mechanisms (Lee et al., 2022). As a result, their concentrations usually peak in the topsoil and then progressively decrease as soil depth increases. Table 2.1 depicts the concentration of nitro-PAHs in the environmental matrix.

Table 2.1. Concentration of nitro-PAHs in the environmental matrix.

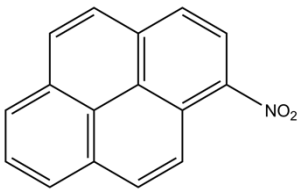
Environmental matrix	Location	Number of nitro-PAHs	The concentration of nitro-PAHs (Σ nitro-PAHs)	References
Air	Athens, Greece	6	2.26–5.86 ng m^{-3}	(Tsakas et al., 2010)
	Xi'an, China	12	0.5–7 ng m^{-3}	(Bandowe et al., 2014)
	Chiang Mai, Thailand	13	0.025–0.72 ng m^{-3}	(Chuesaard et al., 2014)
	Birmingham, United Kingdom	5	0.078–11.7 ng m^{-3}	(Alam et al., 2015)
	Oregon, USA	5	0–0.001 ng m^{-3}	(Lafontaine et al., 2015)
	Longyearbyen, Svalbard	22	2–7.8 ng m^{-3}	(Drotikova et al., 2020)
	Bhagwan Talkies crossing, Agra, India		26.1± 25.9 ng m^{-3}	(Verma et al., 2022)
Soil	Roadside soil of Catania, Italy	9	4–5.2 ng g^{-1}	(Guidi et al., 2012)
	Surface soils of Xi'an, central China	11	29–158 ng g^{-1}	(Wei et al., 2015)
	Pham Van Dong, Hanoi, Vietnam	11	29–158 ng g^{-1}	(Pham et al., 2015)
	Surface soil samples of Yangtze River Delta, China	4	0.4–4.6 ng g^{-1}	(Cai et al., 2017)
	Haidian, China	10	3.61–5.12 ng m^{-3}	(Zhang et al., 2020)
	Kathmandu, Pokhara, Birgunj, and Biratnagar of Nepal	15	396–2530 ng g^{-1}	(Yadav & Devi, 2021)
	Kosetice, Europe	18	0.31 ± 0.23 ng g^{-1}	(Wietzoreck et al., 2022)
Water	Luchuan River, China	6	19.7 ng L^{-1}	(Hung et al., 2012)
	Asano River, Japan	5	604 ng L^{-1}	(Chondo et al., 2013)
	Taige Canal, Changzhou City, China	15	14.7–235 ng L^{-1}	(Kong et al., 2023)

2.2. Chemical Structure and Classification of nitro-PAHs

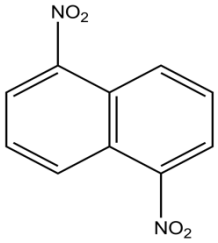
Nitro-PAHs consist of conjugated aromatic hydrocarbon rings (benzene rings) forming a complicated polycyclic structure. Additionally, these compounds contain one or more nitro groups attached to their benzene rings (Sarma et al., 2024). The number of benzene rings in nitro-PAHs is two to many, which are organized in various configurations within the molecules. Nitro-PAHs are classified according to the number of benzene rings they contain. Low Molecular Weight (LMW) nitro-PAHs, which include two or three rings, and High Molecular Weight (HMW) nitro-PAHs, which contain four or more rings (Lee et al., 2022). Examples of LMW nitro-PAHs include 1-nitronaphthalene, 2-nitrofluorene, 1,6-dinitropyrene, 1,5-dinitronaphthalene, 2,3,5-trinitronaphthalene, and 2-nitroanthracene. In contrast, HMW nitro-PAHs include compounds such as 1-nitrobenzo[e]pyrene, 6-nitrobenzo[a]pyrene, and 7-nitrobenzo(ghi)perylene. The chemical structures of various nitro-PAHs are illustrated in Fig. 2.1.



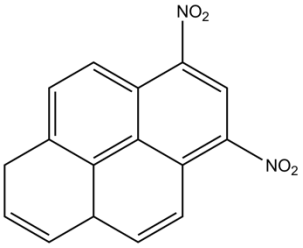
1-nitronaphthalene



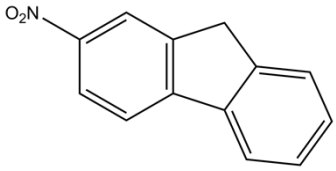
1-nitropyrene



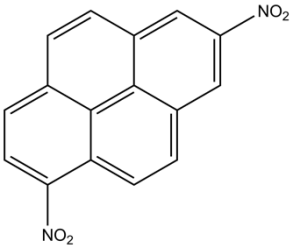
1,5-dinitronaphthalene



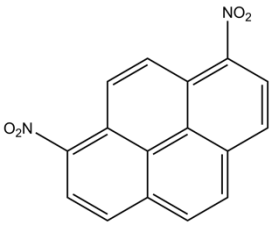
1,3-dinitropyrene



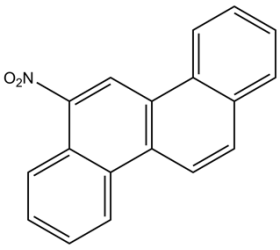
2-nitrofluorene



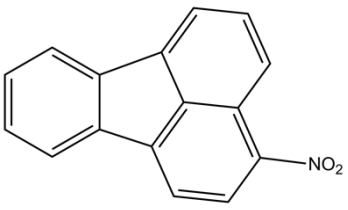
1,6-dinitropyrene



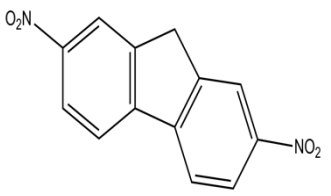
1,8-dinitropyrene



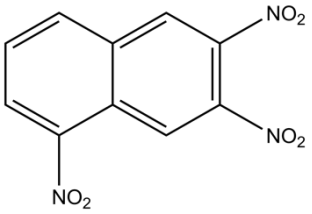
6-nitrochrysene



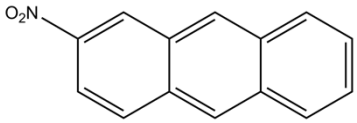
3-nitrofluoranthene



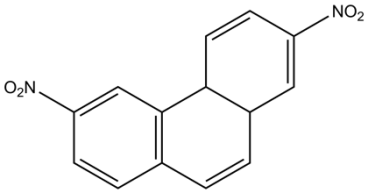
2,7-dinitrofluorene



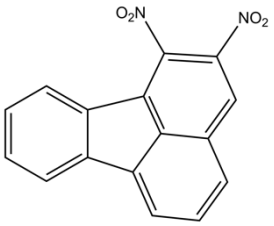
2,3,5-trinitronaphthalene



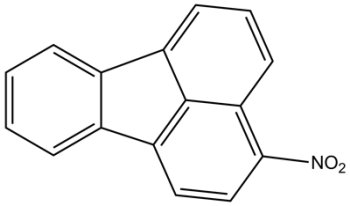
2-nitroanthracene



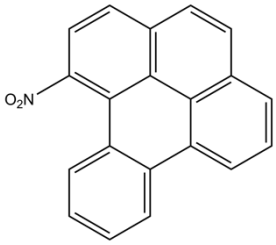
2,6-dinitrophenanthrene



1,2-dinitrofluoranthene



3-nitrobenzo[e]fluoranthene



1-nitrobenzo[e]pyrene

Fig. 2.1. Chemical structure of some specific nitro-PAHs.

The distinctive properties of nitro-PAHs arise due to the presence of NO_2 functional groups attached to the PAH structure. This structural modification, which differentiates nitro-PAHs from their parent PAHs, influences their chemical behavior, environmental fate, and toxicity. Given the classification of nitro-PAHs into LMW and HMW based on ring count, these molecular characteristics further shape their reactivity and persistence in the environment. The key physicochemical properties of nitro-PAHs are described below.

2.2.1. Solubility of nitro-PAHs and Environmental Implications

Solubility specifies the capacity of a material, i.e. solute, to dissolve in another substance, usually a liquid, i.e. solvent, to produce a homogenous combination known as a solution under

a specific temperature and pressure (Singh et al., 2021). Nitro-PAHs are sparingly soluble in water therefore they usually occur in larger quantities in soil or sediment in comparison to water (Stewart et al., 2010). They are readily soluble in organic solvents like acetone, dichloromethane, benzene, hexane, etc (Hayakawa, 2022). For example, 1-nitronaphthalene and 1-nitropyrene are soluble in solvents such as benzene and chloroform but have low solubility in water. Similarly, 2-nitrofluorene and 6-nitrochrysene are soluble in organic solvents like acetone and ethanol but also have low solubility in water (IARC, 2024). The solubility of nitro-PAHs in water drops with increasing molecular size. For example, at 25 °C 1-nitronaphthalene has a water solubility of 9.18 mg/L, but 1-nitropyrene has a far lower solubility of 1.18 x 10^{-2} mg/L (Pubchem, 2024). Environmental factors such as pH, pressure, and temperature can all impact nitro-PAH solubility. Higher temperatures often increase solubility, however severe pH levels can change solubility. Overall, nitro-PAH solubility is affected by its chemical structure, physical properties, and the solvent nature. Given that solubility governs the mobility and bioavailability of nitro-PAHs, it directly influences their environmental persistence and toxicological impact. Therefore, understanding these dynamics is essential for accurately assessing their ecological and health risks.

2.2.2. Melting Points of nitro-PAHs and their Structural Influence

The melting point of a substance is the temperature at which it transforms from solid to liquid at standard atmospheric pressure (Abdullah et al., 2024). In general, higher molecular weight nitro-PAHs often have higher melting points because of stronger molecular interactions and stronger intermolecular forces (Katritzky et al., 2001; Slovokhotov et al., 2004). For instance, the melting point of 1-nitronaphthalene, which is composed of a naphthalene core with a single nitro group, is 61.5 °C (Fanucchi et al., 2004). In contrast to the parent PAH, naphthalene, which melts at about 80 °C, the nitro group raises the molecule's melting temperature by increasing its polarity and promoting intermolecular interactions such as hydrogen bonding and dipole-dipole interactions. Similarly, 1-nitropyrene, a complex nitro-PAH containing four aromatic rings and one nitro group, has a melting point of 155 °C (IARC, 2024). In comparison to prior compounds, its larger molecular weight and bulkiness lead to enhanced intermolecular interactions, raising its melting point even more. Furthermore, substituent nitro-functional groups can affect the melting point of nitro-PAHs. For example, 1,6-dinitropyrene, which has two nitro groups, has a much higher melting point of 310 °C than 1-nitropyrene. The cumulative impact of molecular weight, chemical structure, and the presence of substituent functional groups adds to the compound's significant rise in melting point.

2.2.3. Boiling Points of nitro-PAHs and the Influence of Intermolecular Forces

Nitro-PAHs have high boiling temperatures due to the existence of intermolecular forces that influence molecular interactions (Idowu et al., 2019; Sun et al., 2020). Intermolecular forces, such as dispersion forces, dipole-dipole interactions, and hydrogen bonding, are important in influencing boiling points (Ogden, 2017). Dispersion forces, caused by transitory variations in electron density inside molecules, attract adjacent molecules, with complex nitro-PAHs experiencing higher dispersion forces (Cortés-Arriagada, 2021; Ikawa et al., 2021). Dipole-dipole interactions, which are common in nitro-PAHs with polar bonds such as the nitro group, contribute to total molecule polarity, influencing intermolecular attractions and boiling temperatures. Nitro-PAHs with more polar functional groups may have higher boiling temperatures because of increased dipole-dipole interactions. While hydrogen bonding is less prevalent in nitro-PAHs than in compounds containing hydroxyl or amino groups, its presence can impact boiling temperatures via weak hydrogen bonds between molecules, especially in nitro-PAHs with hydrogen bond donors or acceptor sites. Nitro-PAHs' boiling temperatures are regulated by their molecular weight and mass (Şahin et al., 2022). Larger size and mass cause stronger Van der Waals forces and total molecule attraction, resulting in higher boiling points. Specific nitro-PAHs, such as 3,9-dinitrofluoranthene, and 2,3,5-trinitrofluoranthene, illustrate this, with more nitro groups corresponding to greater boiling points (Kielhorn et al. 2003). Similarly, the boiling point of 1,3-dinitropyrene is 224 °C, but 1,2,4-trinitrofluoranthene has a higher boiling point of 615 °C due to the additional nitro group.

2.2.4. Vapor Pressure of nitro-PAHs: Structural and Environmental Influences

Nitro-PAHs have a broad range of vapor pressures, which are primarily determined by their molecular structure, specifically the number of benzene rings and the presence of nitro groups. Vapor pressure is the equilibrium pressure exerted by a gaseous material in equilibrium with its condensed phases at a specific temperature (Prodan et al., 2024). There is an inverse relationship between the number of benzene rings and vapor pressure. For example, 1-nitronaphthalene, containing two benzenes has a vapor pressure of 1.54×10^{-2} mmHg, 1-nitrofluorene with three rings has a vapor pressure of 9.7×10^{-5} mmHg, and 2-nitrofluoranthene containing four benzene rings has a vapor pressure of 9.9×10^{-7} mmHg (Pubchem, 2024). Also, the nitro group influences the vapor pressure of nitro-PAHs due to increased molecular polarity,

making the molecule less volatile (Barrado et al., 2013). In comparison to pyrene, which has a vapor pressure of 6.0×10^{-4} mmHg, 1-nitropyrene has a lower vapor pressure of 4.4×10^{-6} mmHg. Similarly, incorporating a nitro group into naphthalene, such as in 2-nitronaphthalene, reduces its vapor pressure from 10.4 to 3.2×10^{-2} mmHg (IARC, 2024). Temperature and pressure also have an impact on nitro-PAH vapor pressure. Higher temperatures often enhance the vapor pressure by giving more energy to overcome intermolecular interactions and shift to the vapor state. On the other hand, lower temperatures, reduce vapor pressure by lowering molecules' kinetic energy and slowing their transition into the vapor phase. Simultaneously, pressure can also affect vapor pressure.

2.2.5. Chemical Reactivity and Environmental Transformation of nitro-PAHs

Nitro-PAH reactivity includes a broad spectrum of chemical reactions and changes that take place under different environmental conditions (Yan et al., 2021). Depending on the particular circumstances, these molecules can undergo complexation, reduction, protonation, and oxidation and produce secondary reactive derivatives, e.g. nitroso and nitro derivatives, which may have unique characteristics from the parent molecules (Kovacic & Somanathan, 2014). These interactions can occur in a variety of environmental compartments, including soil, water, air, and biological systems, and have a substantial impact on the behavior, mobility, and possible risk caused by nitro-PAHs (Idowu et al., 2019; Yadav & Devi, 2021). Nitro-PAH oxidation reactions, such as hydroxylation and epoxidation, are notable (Peng et al., 2023; Penning et al., 2022). However, due to the electron-deficient character of the aromatic ring with the nitro substituent, nitro-PAHs are less sensitive to oxidation by various chemical oxidants. Nonetheless, the superoxide anion radical's nucleophilic reactivity is acknowledged, indicating a possible function in the oxidation of nitro-PAHs, particularly when chemically produced (Fukuhara & Miyata, 1995). Reduction processes can produce more bioavailable chemicals, whereas complexation reactions can alter nitro-PAHs sorption on soil particles or absorption by plants and organisms. These derivatives changed chemical reactivities, solubilities, or environmental behaviors, which have affected their persistence and environmental concerns (Falciglia et al., 2016; Zhang et al., 2016). When examining the possible effects of nitro-PAHs on human health and the environment, the reactivity of these compounds must be considered. Table 2.2. provides a comprehensive overview of the physicochemical properties of some selected nitro-PAHs.

Table 2.2. Physicochemical properties of nitro-PAHs (Adopted from (Sarma et al., 2024) with permission).

Sl. No	Nitro-PAHs	Color	Molecular formula	Molecular weight (g/mol)	Boling Point (°C)	Malting Point (°C)	Vapor Pressure [mmHg]	Density (20 °C)
1	1-nitronaphthalene	Pale yellow solid	$C_{10}H_7NO_2$	173.17	304-305 °C	61 °C	4.8×10^{-4}	1.33
2	1,5-dinitronaphthalene	Yellowish-white or light-yellow solid or light-yellow crystalline powder	$C_{10}H_6N_2O_4$	218.17	358.84 °C	138-141 °C	4.28×10^{-6}	1.58
3	2,3,5-trinitronaphthalene	Yellow crystalline solid	$C_{10}H_5N_3O_6$	263.16	286-287 °C	160-162 °C	4×10^{-3}	1.72
4	2-nitrofluorene	Cream-colored solid	$C_{13}H_9NO_2$	211.22	350.9 °C	158 °C	9.54×10^{-6}	1.18
5	2,7-dinitrofluorene	Yellow crystalline solid	$C_{13}H_8N_2O_4$	256.21	399.45 °C	330-334 °C	1.9×10^{-7}	1.32
6	2-nitroanthracene	Yellow-colored solid	$C_{14}H_9NO_2$	223.23	423.9 °C	232.76 °C		1.31
7	2,6-dinitrophenanthrene	Yellow to orange crystalline solid	$C_{14}H_8N_2O_4$	268.22	489.03 °C	207.48 °C		1.61
8	3-nitrofluoranthene	Pale yellow to light brown crystalline solid.	$C_{16}H_9NO_2$	247.25	390.29 °C	157-159 °C	1.04×10^{-7}	1.16
9	1-nitropyrene	Yellow solid or gold solid	$C_{16}H_9NO_2$	247.25	390.29 °C	155 °C	6×10^{-8}	1.16
10	1,3-dinitropyrene	Yellow-orange crystalline solid	$C_{16}H_8N_2O_4$	292.24	434.19 °C	274–276 °C		1.28
11	1,6-dinitropyrene	Yellow-colored solid	$C_{16}H_8N_2O_4$	292.24	434.19 °C	300 °C	9.1×10^{-9}	1.28
12	1,8-dinitropyrene	Yellow to orange-colored solid	$C_{16}H_8N_2O_4$	292.24	434.19 °C	>300 °C	9.1×10^{-9}	1.28
13	1,2-dinitrofluoranthene	Yellow crystalline solid.	$C_{16}H_8N_2O_4$	292.246	548.3±23 °C	279.12 °C	9.1×10^{-10}	1.61
14	1,2,4-trinitrofluoranthene	Light brown crystalline solid	$C_{16}H_7N_3O_6$	337.24	615.2±40.0 °C	305.13 °C		1.71
15	6-nitrochrysene	Yellow, orange-yellow, or chrome-red crystalline solid	$C_{18}H_{11}NO_2$	273.36	416.31 °C	215 °C	1×10^{-8}	1.21
16	3-nitrobenzo[e]fluoranthene	Orange crystals solid	$C_{20}H_{11}NO_2$	297.3	533.5±19.0 °C	211-212 °C	3.1×10^{-10}	1.41
17	1-nitrobenzo[e]pyrene	Orange crystals solid	$C_{20}H_{11}NO_2$	297.3	533.5±19.0 °C	250-250.5 °C	1.1×10^{-9}	1.30

2.3. Detection and Monitoring Challenges of nitro-PAHs in Environmental Matrices

Detecting and monitoring nitro-PAHs from environmental sources is difficult due to their complexity in environmental matrices, where they frequently coexist with PAHs and related derivatives. Under different chromatographic circumstances, these chemicals co-elute with nitro-PAHs and are often found in significantly higher quantities, making direct investigation impossible (Cochran et al., 2012; Dušek et al., 2002). A thorough sample cleaning and prefractionation are tedious but essential for nitro-PAHs trace analysis. Therefore, detecting and monitoring nitro-PAHs in air, soil, and water necessitates a multifaceted method that incorporates extraction, detection, and quantification techniques.

2.3.1. Sampling Techniques for nitro-PAHs in Different Environmental Matrices

Usually, nitro-PAHs sampling procedures differ based on the environmental matrix. Various techniques are used in air sampling, including high-volume air samplers with filters, electrostatic precipitators with impact stages, and filter baghouses that have been specifically designed (Kielhorn et al., 2003; Tutino et al., 2016). These techniques enable the collection of particulate matter and gas-phase nitro-PAHs over defined sampling periods. Several absorbing media are used to absorb nitro-PAHs from ambient air including Tenax-GC solid adsorbent, polyurethane foam (PUF), and filters (Kumari & Lakhani, 2018; Zimmermann et al., 2013). After collection, samples are wrapped in aluminum foil and kept at -20 °C until analysis (Fomba et al., 2024). Simultaneously, Cryogenic sampling, adsorbent samplers with materials such as XAD-2 and PUF, and dilution tunnel techniques are routinely used to collect nitro-PAHs in diesel exhaust vapor and particle phases (Heeb et al., 2008; Lucas et al., 1991).

Dissolved and particle phase nitro-PAHs are separated during the sampling of water using filtration procedures. Grab samplers are used to retrieve sediment samples from various depths, whilst water samples are collected in amber glass jars and stored at -20 °C to preserve sample integrity (Kong et al., 2023).

For soil sampling surface and deeper soil layers are systematically collected and allowed to be air-dried, ground, and sieved before storage (Wietzoreck et al., 2022). To avoid contamination and maintain the integrity of the samples collected, soil samples are treated with extreme caution. To some extent, the soil samples are dried by using drying agents like Na_2SO_4, and

diatomaceous earth (Siudek & Ruczyńska, 2021; Zhang et al., 2014). Soils can be air-dried or freeze-dried before analysis, depending on the storage method.

2.3.2. Extraction Methods for nitro-PAHs from Environmental Matrices

Extraction methods play an essential role in the detection of nitro-PAHs, providing a variety of approaches for purifying these chemicals from complex environmental matrices. The most often used techniques include liquid-liquid extraction (LLE), solid-phase extraction (SPE), soxhlet extraction, pressured solvent extraction, supercritical fluid extraction, and QuEChERS (Andrade-Eiroa et al., 2010; Garcia-Alonso et al., 2012).

2.3.2.1. Liquid-liquid Extraction

Liquid-liquid extraction, commonly known as solvent extraction, is a traditional method of separating solutes by transferring them from one solvent to another. This approach entails mixing the sample with an immiscible or slightly miscible solvent, followed by phase separation (Su et al., 2017). The addition of salt to create a salting-out effect, as well as pH adjustments, can improve the effectiveness of liquid-liquid extraction (Gezahegn et al., 2019). The extraction efficiency is influenced by a variety of characteristics such as shaking speed and periods, pH, solvent polarity, ionic strength, solvent-to-sample ratio, and temperature. Ultrasound-assisted extraction (UAE) is frequently used to speed up the procedure and boost efficiency (Pérez & Albero, 2023; Shen et al., 2023). Acetone, dichloromethane, and n-hexane are common solvents for liquid-liquid extraction of nitro-PAHs (Hayakawa, 2022).

Although liquid-liquid extraction is time-saving, it may not give satisfactory results for some nitro-PAHs, and co-existing compounds may be removed, necessitating purifying measures before analysis. Furthermore, this approach often requires a substantial volume of organic solvent, which raises environmental and health problems as well as operator exposure risks (Almohasin et al., 2023).

2.3.2.2. Liquid-phase Microextraction (LPME)

Liquid-phase microextraction (LPME) is a precise and effective sample pretreatment process that extracts nitro-PAHs from a variety of matrices before instrumental analysis (Guiñez et al., 2018; Shirani et al., 2023). This approach is based on the equilibrium distribution of analytes between an aqueous sample and a small volume of hydrophobic organic solvent, combining extraction and concentration processes for increased efficiency (Dugheri et al., 2020; Prosen, 2014). The extractant phase can be immersed in the sample (direct immersion liquid-phase

microextraction) or suspended above it (head-space liquid-phase microextraction) (Shirani et al., 2023). Stirring the sample solution helps to move nitro-PAHs from the aqueous phase to the organic extractant. Because of the limited amount of extractant in comparison to the sample solution, the sample volume to extractant volume ratio is high, resulting in significant enrichment factors.

This technique can be categorized into three sub-divisions: dispersive liquid-liquid microextraction (DLLME), single-drop microextraction (SDME), and solidification of floating organic drop-dispersive liquid-liquid microextraction (SFO-DLLME). Each of the categories has its unique procedures and applications (Câmara et al., 2022; Yamini et al., 2019).

DLLME is a three-solvent-based approach that efficiently extracts and preconcentrates nitro-PAHs with microliters of organic solvent. DLLME contains three phases: the extraction solvent (acceptor phase), the dispersive solvent, and the aqueous phase (donor phase, where the analyte is found) (Tseng et al., 2014; Wang et al., 2022). A small amount of organic solvent combined with a dispersive solvent that is miscible with the extractant and aqueous phases is quickly applied to the sample in this process (Liang et al., 2017). The equilibrium state is quickly attained due to the relatively large area between the acceptor phase (extractor solvent) and the donor phase (aqueous sample), making the isolation time very fast. Several studies have highlighted that utilized DLLME to effectively extract nitro-PAHs from various matrices (Liu et al., 2024; Soriano et al., 2024). Borgous et al. (2017) used this approach to extract six nitro-PAHs from a water matrix (Borges et al., 2018). Guiñez et al. (2018) used a solvent-based de-emulsification DLLME to enrich nitro-PAHs and oxygenated PAHs in water samples, achieving enrichment factors of 191-200 (Guiñez et al., 2018).

In SDME, a micro drop of the extraction solvent is suspended from a microsyringe tip and submerged in the aqueous sample (Gupta et al., 2025; Sanchís et al., 2017). In HS-LPME (headspace liquid-phase microextraction), the drop can be suspended over the sample solution (Mogaddam et al., 2019; Albarri et al., 2024). When the solution is agitated, nitro-PAHs move from the aqueous phase to the organic droplet. Upon attaining equilibrium, the droplet is withdrawn into the microsyringe and injected into a chromatographic apparatus for examination (Hayakawa, 2022).

Similarly, in SFO-DLLME process uses an organic solvent with a density less than water and a melting point close to room temperature (10-30 °C) (Atakol et al., 2025; Hryniewicka et al., 2019). A disperser is used to disperse the extractant into the sample solution during extraction.

The analytes migrate into the extractant, which floats on the solution's surface following vortex mixing and centrifugation (Leong & Huang, 2008). Cooling causes the extractant to solidify, which is then collected, melted, and examined.

2.3.2.3. Soxhlet Extraction for nitro-PAHs: Methodology and Applications

Soxhlet extraction is a well-known process for extracting nitro-PAHs from a variety of environmental matrices including airborne particles, sediments, soil, and food samples, utilizing solvent reflux and siphoning principles to achieve high extraction efficiency (Lara et al., 2023; Wang et al., 2025). This approach frequently requires washing the solid sample with a new solvent to ensure the analytes are fully extracted. The procedure normally lasts 8-12 hours, allowing for a thorough extraction of the nitro-PAHs. Li et al. (2015) successfully extracted the nitro-PAHs in an air sample with the help of Soxhlet extraction (Li et al., 2015). They used polyurethane foam to collect gaseous phase nitro-PAHs, which were subsequently Soxhlet extracted for 8 hours using 150 mL of a hexane-acetone combination (1:1, v/v). This method ensured that the nitro-PAHs were extracted completely from the PUF samples. The solvent containing the extracted analytes was concentrated and purified on a silica gel and alumina column to eliminate impurities and improve the analytical findings (Li et al., 2015). Different solvents, such as dichloromethane and acetonitrile, have been used in Soxhlet extraction to maximize the recovery of nitro-PAHs from different matrices.

2.3.2.4. QuEChERS Approach for nitro-PAHs Extraction: Methodology and Applications

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach is a flexible and effective sample preparation methodology used to extract nitro-PAHs from complex matrices (Albinet et al., 2013; Jing et al., 2023). Originally QuEChERS approach was designed for pesticide residue analysis in food, now it has been modified for a variety of other applications due to its cost-effectiveness, and high efficiency (Muhammad et al., 2017). The extraction of nitro-PAHs in the QuEChERS approach begins with the homogenization of the sample, which can be soil, sediment, food, or any environmental matrix (Rivoira et al., 2023). A portion of the homogenized material is put in a centrifuge tube, and an extraction solvent, usually acetonitrile, is added. Salts ($MgSO_4$ and NaCl) are added to the tube after the solvent is introduced. These salts aid in phase separation by pushing analytes into the organic solvent phase. The tube is then forcefully shaken to enable proper mixing and effective extraction of nitro-PAHs from the sample matrix into acetonitrile (Deng & Chan, 2017; Sonego et al., 2022). Deng and Chan

(2017) have successfully used the QuEChERS-based method to extract 1-nitropyrene and 2-nitrofluorene from rice grains and vegetables. This process involves adding 10 mL of acetonitrile to 10 g of homogenized rice or vegetable samples, followed by 30 minutes of ultrasonication. Subsequently, add 1 g of NaCl and 4 g of anhydrous $MgSO_4$, mix thoroughly, and centrifuge. To clean 1 mL of the supernatant, use 30 mg of PSA sorbent and 150 mg of anhydrous $MgSO_4$ (Deng & Chan, 2017). The solution is vortexed and centrifuged again, and the resultant supernatant is sent for analysis. Albinet et al. 2014 used a QuEChERS approach to extract 32 nitrated and 32 oxygenated PAH derivatives from organic aerosols, demonstrating the technology's adaptability and efficacy across a wide range of samples (Albinet et al., 2014).

2.3.2.5. Solid-Phase Microextraction for nitro-PAHs Analysis: Principles and Advances

Solid-phase microextraction is a cost-effective and eco-friendly sample preparation approach used to extract and analyze nitro-PAHs (Adeniji et al., 2025; Wang et al., 202). This process combines sampling, extraction, and concentration in a single step, which increases efficiency and saves time. Solid-phase microextraction works on the principle of partitioning and adsorption, with a fiber coated with a sorbent substance exposed to the sample matrix either directly (direct immersion Solid-phase microextraction) or in the headspace above the sample (Jalili et al., 2020; Nolvachai et al., 2023). Because of the low volatility of nitro-PAHs, straight immersion Solid-phase microextraction is frequently the favored technique of extraction.

The Solid-phase microextraction process begins with selecting a suitable fiber coating. Polydimethylsiloxane, polyacrylate, divinylbenzene, and carboxen are common coatings that are appropriate for extracting volatile, semi-volatile, polar, and non-polar compounds, with varying affinities for different analytes (Agatonovic-Kustrin et al., 2023; Nascimento et al., 2019). Fibers have a high affinity for aromatic and nitro-PAHs. The fiber is exposed to the sample for a predeterminate time, allowing the nitro-PAHs to partition between the sample matrix and the fiber coating. During the extraction phase, the sample matrix and fiber coating reach an equilibrium (Doong et al., 2000; Gong et al., 2022). Temperature, extraction period, and agitation are all factors that can impact extraction efficiency. Usually, the sample is stirred to increase the contact between the analytes and the fiber coating, which improves extraction efficiency. The exposure period varies depending on the number of nitro-PAHs and the intricacy of the matrix, although it typically ranges between a few minutes and an hour (Psillakis & Kalogerakis, 2001).

Once the extraction is finished, the fiber is withdrawn into the Solid-phase microextraction holder and sent to an analytical instrument, which is often gas chromatography or liquid chromatography combined with mass spectrometry. Kong et al. (2019) evaluated the extraction efficiency of nitro-PAHs using five commercial fibers coated with PDMS/DVB, PDMS, and PA at various specifications (Kong et al., 2019). They revealed that a 65 μm PDMS/DVB fiber is optimal for aqueous extraction. In recent years, new coating materials for Solid-phase microextraction have been developed, increasing its possibilities. Sol-gel-based metal-organic framework zeolite imidazolate framework-8 fibers, zinc(II)-based metal-organic nanotubes, and porphyrinic zirconium metal-organic framework have all been used as Solid-phase microextraction fiber coating materials to adsorb nitro-PAHs in aqueous samples (Kong et al., 2019). These advancements increase Solid-phase microextraction's application and efficiency, transforming it into a versatile and potent instrument for environmental and analytical chemistry.

2.3.3. Analytical Techniques for nitro-PAHs Detection in Environmental Samples

The principal techniques for evaluating nitro-PAHs qualitatively and quantitatively in diverse environmental matrices are gas chromatography coupled with mass spectrometry (GC-MS) and high-performance liquid chromatography coupled with either fluorescence detection or mass spectrometry (HPLC-FLD/MS) (Wang et al., 2021; Wen et al., 2023). While electrochemical techniques were previously used, their effectiveness for trace detection was restricted due to the low quantities of nitro-PAHs in samples. Because of their sensitivity and specificity, mass spectrometry techniques have emerged as popular choices, notably tandem mass spectrometry (MS/MS) and high-resolution mass spectrometry (Lung & Liu, 2015; Song et al., 2024). Because of the weak fluorescence of nitro-PAHs, fluorescence detection is sensitive but needs pre-column or online fluorescence derivatization. These analyses employ diverse mass spectrometry methods, including GC-NCI-MS, GC-ECD, GC-MS with electron ionization, and different HPLC-MS combinations, as well as mass analyzers such as quadrupole mass spectrometers (QMS), orbitrap analyzers, and time-of-flight (ToF) analyzers (Kawanaka et al., 2007; Lee et al., 2022). Alternative methods such as gas chromatography with atmospheric pressure chemical ionization (GC-APCI) and comprehensive two-dimensional gas chromatography (GCGC) have been developed to overcome the limitations of electrochemical and fluorescence detectors, significantly improving nitro-PAHs analysis in complex environmental samples.

2.4. Toxicological Effects and Human Exposure to nitro-PAHs

Nitro-PAHs have toxicological effects that include the possible impact on human health and the environment. These effects extend to aquatic ecosystems and soil microbial communities. These chemicals have a wide range of hazardous consequences due to their physicochemical properties and endurance (Cao et al., 2022; Feng et al., 2025). Nitro-PAHs cause genotoxicity, carcinogenicity, mutagenicity, oxidative stress, endocrine disruption, and neurotoxicity in a variety of species, including terrestrial invertebrates, and humans (Luderer, 2024; Sarma et al., 2024). They also influence aquatic and avian species, causing ecotoxicity (Manzetti, 2012). Human exposure to nitro-PAHs can come from a variety of sources, including food consumption, tobacco smoking, environmental pollution, and occupational activities (Bandowe & Meusel, 2017). Individuals who are exposed to exhaust fumes, such as mechanics, street sellers, and motorcycle riders, as well as those who work in mining and metallurgy, may be exposed to nitro-PAHs (Hendryx et al., 2020; Verma et al., 2022). Human exposure to nitro-PAHs occurs by ingestion, inhalation, and skin contact (Yaffe et al., 2001; Zhang et al.,2023). The severity of adverse effects is influenced by the mode, duration, and dose of exposure. A summary of the health impacts associated with nitro-PAH exposure is provided in Table 2.3. The toxicological effects of nitro-PAHs, including their potential impact on human health are cited below.

2.4.1. Genotoxic Mechanisms of nitro-PAHs

Nitro-PAHs pose a serious concern in environmental and public health contexts because of their propensity to cause genotoxicity in living organisms. Nitro-PAHs' genotoxic characteristics have been studied since the late 1970s (Clergé et al., 2019; Möller, 1994). These chemicals have been found to have impacts through various mechanisms, including covalent DNA binding, mutation induction, interference with DNA repair systems, and disruption of cellular signalling networks (Asare, 2009). Ames and coworkers published one of the first signs of nitro-PAH genotoxicity in 1975. They found that numerous nitro-PAHs were mutagenic in *Salmonella typhimurium*, and ultimately it leads to genetic alterations. Nitro-PAHs' genotoxic effects have been extensively studied in bacterial models as well as mammalian cell cultures. Nitro-PAHs' interaction with DNA is one of the key mechanisms by which they cause genotoxicity (Wójcik et al., 2022). These chemicals can covalently link with DNA molecules, forming DNA adducts (Ewa & Danuta, 2017; Fu et al., 1994). These adducts can disrupt DNA replication and transcription, potentially causing mutations and genomic instability.

Furthermore, nitro-PAH-induced DNA damage can elicit cellular responses such as DNA repair mechanisms; but severe or chronic damage may overwhelm these repair systems, resulting in the accumulation of mutations and genetic changes. Oxidative stress is another significant mechanism through which nitro-PAHs exert their genotoxic effects. Nitro-PAHs have the potential to generate ROS within cells, causing oxidative damage to DNA, proteins, and lipids (Shang et al., 2017; Xia et al., 2013). ROS-induced DNA damage by breaking the DNA strands and base oxidation, which leads to genomic instability, raising the possibility of mutations (Poetsch, 2020).

2.4.2. Oxidative Stress and Inflammatory Responses Induced by nitro-PAHs

Oxidative stress and inflammation triggered by nitro-PAHs pose significant threats to biodiversity (Neophytou et al., 2014). Oxidative stress, defined as an imbalance between the formation of ROS and the body's ability to neutralize them with antioxidants, is a significant consequence of nitro-PAH exposure (Piao et al., 2023; Shankar & Mehendale, 2014). These substances can produce ROS directly or activate metabolic pathways to produce reactive intermediates that cause oxidative damage to cellular components such as lipids, proteins, and DNA (Libalova et al., 2018; Xia et al., 2013). Oxidative damage can cause cellular malfunction, signaling pathway disruption, and even cell death (Afzal et al., 2023; Olufunmilayo et al., 2023). Nitro-PAH exposure has also been linked to inflammation (Lee et al., 2022). These contaminants were reported to cause inflammation in the body by activating pro-inflammatory signaling pathways and prompting immune cells to produce inflammatory mediators (Peng et al., 2023; Xu et al., 2018). The immune system responds to nitro-PAHs by recruiting immune cells to the site of exposure, where they release cytokines, chemokines, and other inflammatory compounds (Øvrevik et al., 2010; Shang et al., 2017). Chronic exposure to nitro-PAHs can cause inflammation, tissue damage, organ malfunction, and the onset of inflammatory conditions. Shang et al. (2017) examined nitro-PAHs' inflammatory effects, especially their potential to trigger pro-inflammatory pathways in human cells. The study found that nitro-PAHs such as 1-nitropyrene and 3-nitrofluoranthene activate signaling pathways including NF-κB and PI3K/Akt, leading to increased production of pro-inflammatory mediators and cytokines (Shang et al., 2017). These data indicate that nitro-PAHs exposure might increase inflammatory responses, resulting in tissue damage and possible health effects. Moreover, Øvrevik et al. (2010) found that particular nitro-PAHs, such as 1-nitropyrene, 3-nitrofluoranthene, and 3-nitrobenzanthrone, can significantly increase inflammation in human cells. These substances were discovered to activate pro-inflammatory pathways and enhance

the generation of inflammatory mediators, resulting in increased inflammation and related health hazards (Øvrevik et al., 2010). Similarly, Tsai et al. (2021) highlighted that 1-nitropyrene increases the production of proinflammatory mediators such as nitric oxide and prostaglandin E2 via iNOS and COX2. It activates the NF-κB pathway and phosphorylates Akt, leading to the generation of proinflammatory cytokines such as IL-1β, IL-6, and TNFα. This causes inflammation due to macrophage overactivation (Tsai et al., 2021).

2.4.3. Carcinogenic Potential of nitro-PAHs and Their Impact on Organisms

Nitro-PAHs pose a significant risk to organisms due to their carcinogenic properties. These substances have been identified as probable carcinogens following extensive studies in laboratory animals and epidemiological data (Hong et al., 2021; Talaska et al., 1996). Nitro-PAHs' carcinogenic activity is due to their ability to undergo metabolic activation within organisms. This metabolic transition produces reactive intermediates that can interact with biological macromolecules such as DNA, proteins, and lipids (Idowu et al., 2019). Furthermore, many nitro-PAHs undergo metabolism, resulting in highly genotoxic chemicals and increasing the risk of cancer formation. The International Agency for Research on Cancer (IARC) has classified nitro-PAHs into four groups based on their carcinogenic potential: Group 1, Group 2A, Group 2B, and Group 3.

3-nitrobenzanthrene, a key member of the nitro-PAHs, has been thoroughly investigated for carcinogenic properties. Landavik et al. (2010) shed light on the complex molecular mechanisms of 3-nitrobenzanthrene and its metabolites, revealing their pernicious effects on cellular homeostasis and genomic integrity (Landvik et al., 2010). Their findings revealed that the intricate interplay between DNA damage, apoptotic signaling, and immune regulation, highlights the multifarious character of nitro-PAH-induced carcinogenesis and provides a compelling narrative on the molecular foundations of cancer initiation and progression. Similarly, Hansen et al. (2007) found that intracellular ROS played a critical role in fuelling the oncogenic cascade initiated by 3-nitrobenzanthrene, underscoring the complex interaction between oxidative stress, DNA damage, and carcinogenesis (Hansen et al., 2007).

Various nitro-PAHs have been shown to cause cancer in animals. 6-nitrochrysene, 3,7- and 3,9-dinitrofluoranthene, 3-nitrofluoranthene, 1- and 4-nitropyrene, 1,3-, 1,6-, and 1,8-dinitropyrene

Table 2.3. The human health impact of specific nitro-PAHs.

Nitro-PAHs	Carcinogenic group	Exposure route	Associated diseases	References
1,5-dinitronaphthalene	None carcinogen	Inhalation	Respiratory irritation, methemoglobinemia, skin irritation, eye irritation, kidney degeneration, and kidney damage	(IARC, 2024)
1-nitropyrene	Group 2A	Inhalation	Chronic obstructive pulmonary disease, adenocarcinoma, asthma, atherosclerosis, emphysema, and hypertension	(Yarmohammadi & Karimi, 2024; Gao et al., 2022)
2,7-dinitrofluorene	Group 2A	Inhalation	Eye, skin, and respiratory tract irritation and develop cancer	(Malejka-Giganti et al., 2008)
1,6-dinitropyrene	Group 2 B	Inhalation and skin contact	Papilloma, histiocytoma, and vulvar neoplasms	(IARC, 2024)
6-nitrochrysene	Group 2 B	Inhalation and skin contact	Liver neoplasms, cell transformation, and mammary neoplasms	(IARC, 2024; Zhang et al., 2023)
1,3-dinitropyrene	Group 2B	Inhalation	Histiocytoma, papilloma, liver neoplasms, and mammary neoplasms	(Misaki et al., 2022)
2-nitrofluorene	Group 2 B	Inhalation and skin contact	Inflammation, irritation, endocrine-disrupting, and liver neoplasms	(Zhou et al., 2022)
1-nitronaphthalene	Group 3	Inhalation	Vomiting, coughing, acute liver and lung infection, irritation, redness, wheezing, nausea, itching, and diarrhea	(Lin et al., 2009; Verschoyle et al., 1993)
3-nitrofluoranthene	Group 3	Inhalation and skin contact	Neoplastic, apoptosis, and necrosis	(Tokiwa et al., 1993)

and 5-nitroacenaphthene are some examples (Kielhorn et al., 2003). In addition, 3-nitroperylene, 2-nitropyrene, 7-nitrodibenz[a,h]anthracene, 2- and 6-nitrobenzo[a]pyrene, 3,6-dinitrobenzo[a]pyrene, and 7-nitrobenz[a]-anthracene, have exhibited carcinogenic effects in experimental animals (IARC, 2024). These substances primarily cause systemic cancers in organs like the mammary gland, the lung, the liver, and the hematological system. Oral exposure to 2-nitrofluorene and 5-nitroacenaphthene resulted in local carcinogenic effects in rats' digestive systems (Möller et al., 1987; Takemura et al., 1974). Skin cancers in mice were seen following dermal exposure to 6-nitrochrysene and 3-nitroperylene (El-Bayoumy et al., 1982).

2.4.4. Impact of nitro-PAHs on Plant Health and Development

Nitro-PAHs have a substantial impact on plant health, as evidenced by their presence in various plant species in urban and suburban contexts (Talaska et al., 1996; Yun et al., 2019). Plants absorb these substances from the soil and air and store them mostly in their roots or transfer them to branches via the xylem (Undersander & Sharpe, 2025; Yun et al., 2019). This accumulation caused phytotoxicity, genotoxicity, oxidative stress, and disruption of nutrient absorption and metabolism (Wang et al., 2023). However, certain plant species show tolerance to this stress by producing enzymes capable of digesting pollutants. For example, Nakajima (1994) reported that Azalea leaves accumulate 1-nitropyrene at concentrations of 0.14 to 2.5 ng/g, whereas tea leaves accumulate 3,6-dinitrobenzo[e]pyrene at concentrations of 0.008 to 1.82 ng/g, obtained from polluted air and soil during plant development (Hasei et al., 2011; Nakajima, 1994). Existing literature revealed that higher concentrations of nitro-PAHs found in particulate matter samples from Rio de Janeiro and Córdoba have been associated with cytotoxic and genotoxic effects on plants, including *Allium cepa* and *Tradescantia pallida* (Yun et al., 2019). Furthermore, exposure to nitro-PAHs such as 1-nitropyrene hinders barley germination and growth, potentially lowering crop yields by causing chromosomal damage and genotoxicity in barley root tip cells (Yun et al., 2019). Plants in a stressful environment produce ROS, such as superoxide radicals ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which cause oxidative damage to membrane lipids, proteins, and nucleic acids and ultimately hinder overall plant growth and development (Hasanuzzaman et al., 2020; Huang et al., 2019).

2.4.5. Impact of nitro-PAHs on Soil Microbial Communities and Ecosystem Dynamics

Nitro-PAHs also negatively impact soil microbial communities, with consequences that extend beyond ecosystems (Cao et al., 2022; Wójcik et al., 2022). Their effects on these communities are multifaceted, including structural alterations, shifts in diversity, and disturbances in metabolic functioning. Nitro-PAH exposure can cause selective pressures to act in favor of some microbial species while disadvantageously affecting others, changing community composition (Bandowe & Meusel, 2017). This reshuffling may promote the dominance of nitro-PAH-tolerant species, disrupting the balance of microbial diversity in soil. Furthermore, nitro-PAHs directly interfere with microbial metabolic processes, reducing enzyme activity and disrupting cellular functioning, impeding critical biochemical pathways necessary for metabolism, respiration, and biosynthesis (Fu, 1990). As a result, the vitality and activity of soil microorganisms may be reduced, limiting their capacity to perform critical roles within soil ecosystems. Although the details investigation of nitro-PAHs toxicity to soil microflora is still in the initial stage, research in liquid culture methods has demonstrated significant inhibitory effects of nitro-PAHs on microbes. Elevated concentrations of nitro-PAHs have been reported to inhibit the development of nitro-PAHs degradable microorganisms such as *Mycobacterium gilvum* LB307T and *Sphingomonas sp* LH128 (Meyer & Steinhart, 2000). Meyer and Steinhart (2000) also revealed that due to exposure nitro-PAHs in PAHs contaminated soil, leads to extended lag phases and slower degradation rates. Nitro-PAHs have also been linked to decreased CO_2 generation during soil incubation and resistance to biotransformation. The appearance of "dead-end" compounds in cell cultures exposed to nitro-PAHs indicates that microbial metabolism and degradation pathways are becoming more complicated (Meyer & Steinhart, 2000). These findings emphasize the problems posed by nitro-PAHs to microbial communities and the fine balance of soil ecosystem dynamics.

2.4.6. Persistence and Bioaccumulation of nitro-PAHs: Environmental and Health Implications

The persistence and bioaccumulation of nitro-PAHs are significant factors in their environmental effect (Huang & Batterman, 2014). Persistence refers to the capacity to withstand deterioration in the environment over time (Bamford et al., 2003; Cousins et al., 2019). Because of their complex chemical structure, low water solubility, and resistance to microbial destruction, nitro-PAHs can remain in a variety of environmental compartments,

including soil, water, and air, for long periods ranging from months to decades. Simultaneously, another crucial component of nitro-PAH activity is bioaccumulation, which occurs when these chemicals accumulate in the tissues of organisms after being absorbed by the environment (Behl et al., 2024; Yu et al., 2019). Because of their lipophilic nature, nitro-PAHs can accumulate in lipid-rich tissues and organs, magnifying their presence in food chains (Nowakowski et al., 2022). As a result, these substances become disseminated among top predators such as fish, birds, and mammals, posing threats to both ecological stability and human health upon ingestion. Onduka et al. (2012) conducted acute toxicity tests of nitro-PAHs across several trophic levels in marine food webs, demonstrating these chemicals' extensive impact on three trophic levels (Onduka et al., 2012). Nitro-PAHs persistence and bioaccumulation have far-reaching repercussions, changing ecological processes, altering community structure, and reducing wildlife health and reproductive performance. Furthermore, bioaccumulated nitro-PAHs might endanger human health if consumed in contaminated food and water. Chronic exposure to nitro-PAHs has been associated with detrimental health consequences, including carcinogenicity, mutagenicity, developmental abnormalities, and reproductive impairments.

2.5. Regulatory Challenges and Advancements in Phytoremediation Strategies for nitro-PAHs

In most cases, hazardous organic pollutants have been regulated through the establishment of permissible environmental limits by international agencies such as the World Health Organization (WHO), the U.S. EPA, and the Occupational Safety and Health Administration (OSHA). For instance, OSHA has set the exposure limit for PAHs at 0.2 mg/m³ of air. However, despite the known toxicity and environmental significance of nitro-PAHs, no clear or standardized exposure limits have been established. This regulatory gap highlights the need for further research and stricter oversight to address the risks associated with these compounds. Effectively mitigating the environmental impact of nitro-PAHs will require the development and implementation of efficient remediation strategies for contaminated sites.

In recent decades many techniques have been developed to rehabilitate soils tainted by PAHs, each displaying varying levels of effectiveness (Falciglia et al., 2016; Sarma et al., 2024). Nevertheless, most physicochemical methods are accompanied by notable disadvantages such as high operational expenditures, labor-intensive, ineffectiveness at low pollutant concentrations, the necessity for extensive infrastructure, and adverse effects of the

surrounding environmental matrix (Sarma et al., 2024). The phytoremediation approach carried out on plants, emerges as a cost-effective, eco-friendly method for alleviating the impact of PAHs on polluted land by the use of plant inherent metabolic capabilities to immobilize, mineralize, and neutralize contaminants (Li et al., 2020; Yasin et al., 2024) (Fig. 2.2). Previous studies have shown that plants can lower the bioavailability of both organic and inorganic pollutants in soil by stabilizing or eliminating them (Hasan et al., 2025; Maske et al., 2025). The main processes involved in nitro-PAHs' phytoremediation encompass plant uptake and metabolism (Li et al., 2020). Some specific plant species are reported to uptake nitro-PAHs (Scheidemann et al., 1998; Yun et al., 2019). Initially, the breakdown of nitro-PAHs is initiated by nitrate reductase, which converts the nitro group into ammonia. Subsequently, cytochrome P450 monooxygenases activate molecular oxygen using electrons from NADPH and insert a single oxygen atom into substrates (Spain, 1995; Kielhorn et al., 2003). These particular enzymes are typically responsible for catalyzing various reactions like hydroxylation, epoxidation, dealkylation, and reductive dehalogenation of aromatic rings, although there have been no reports of them catalyzing the opening of aromatic rings (Kiel & Engesser, 2015; Molina & Segura, 2021). Following that the aromatic rings are oxidized by adding two hydroxyl groups with the help of dioxygenase enzymes (Kaur et al., 2023). The subsequent phases of degradation involve various oxidoreductases like peroxidases, as well as carboxylesterases (Siddiqui & Dahiya, 2022). These enzymes work together to convert contaminants into less hydrophobic compounds, thereby enhancing their reactivity, which is categorized as Phase I of the detoxification process. Consequently, in Phase II, the contaminants undergo conjugation reactions with a variety of plant primary metabolites such as proteins, amino acids, organic acids, glutathione, polysaccharides, lignin, and peptides (Molina & Segura, 2021). This conjugation step leads to the formation of hydrophilic compounds. The catalysis of these conjugation reactions is facilitated by an array of transferase enzymes, which encompass enzymes like glutathione S-transferase (GST) and glycosyltransferases. These conjugate compounds are contained within a cell without inducing any observable pathological manifestations because their toxicity is comparatively reduced due to above mention reaction (Hernández-Vega et al., 2017; Molina & Segura, 2021). It has been observed that in certain circumstances, over 70% of organic pollutants are absorbed by plants and stored in the form of conjugated compounds in cell walls and vacuoles (Kouche et al., 2025; Zhang et al., 2017).

An exemplary instance can be seen in the phytoremediation of 2,4,6-trinitrotoluene (TNT). Within plants, TNT undergoes detoxification via biochemical reaction, becoming linked to plant metabolites, and stored in plant tissues or polymers rather than being broken down into carbon dioxide and nitrogen. This remediation process generated several reduction byproducts like 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2-hydroxylamino-4,6-dinitrotoluene, and 4-hydroxylamino-2,6-dinitrotoluene have been detected in numerous plant species (Hoehamer et al., 2006). The majority of these byproducts are retained in the roots, with their levels generally surpassing those of TNT itself, while lesser quantities accumulate in the stems and leaves. It was also reported that an aquatic plant, i.e. *Myriophyllum aquaticum* oxidized TNT. The oxidative byproduct of TNT includes 2-amino-4,6-dinitrobenzoate, 2-N-acetoxyamino-4,6-dinitrobenzaldehyde, 2,4-dinitro-6-hydroxybenzyl alcohol, and 2,4-dinitro-6-hydroxytoluene (Tront & Saunders, 2006). The latter two compounds are predicted to arise from ring hydroxylation accompanied by the elimination of a nitro group, a finding of notable importance since derivatives with reduced nitro groups are more prone to subsequent degradation processes.

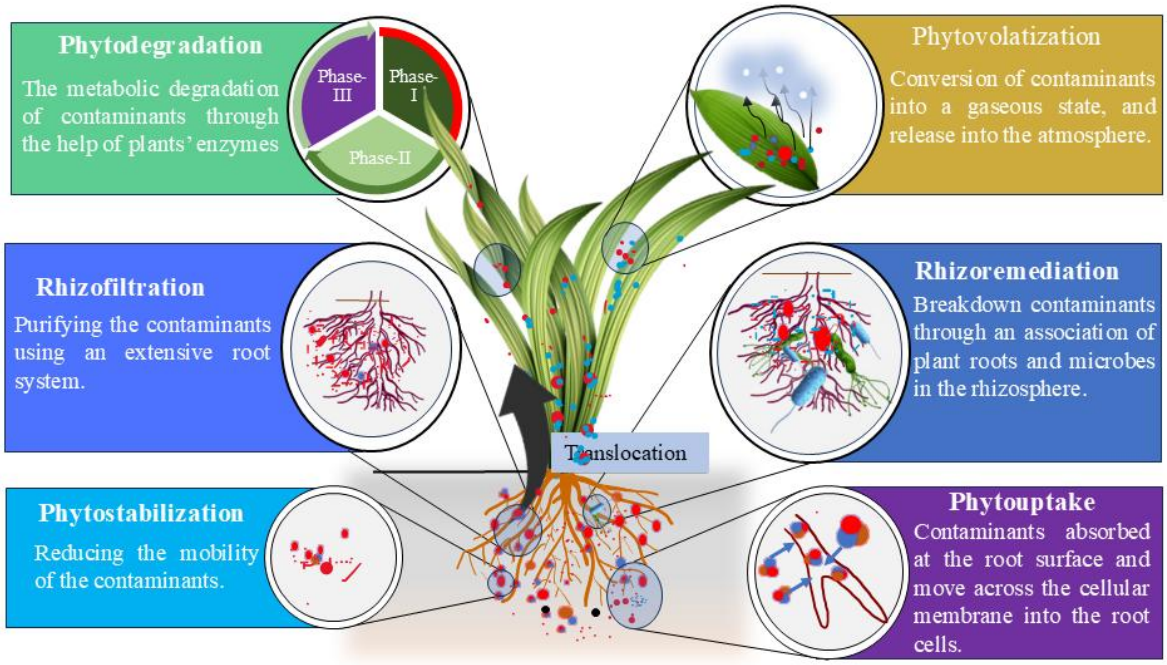


Fig. 2.2. Mechanism of phytoremediation. Plants absorb, transform, and mitigate organic contaminants. The mechanisms began with phytouptake, followed by phytodegradation, where pollutants are enzymatically broken down into less toxic forms, and phytovolatilization, where they are released as gases into the atmosphere. rhizoremediation involves microbial

interactions in the rhizosphere to degrade pollutants. The overall process depends on translocation of the pollutants within the plant tissues.

2.6. Microbial Degradation of nitro-PAHs

Microbial degradation of nitro-PAHs is an essential process that utilizes the metabolic capabilities of various bacteria and fungi to convert these hazardous compounds into less harmful substances. This process is initiated by specific enzymes, such as nitroreductases, dioxygenases, and monooxygenases, which are produced by certain bacteria. These enzymes play a key role in breaking down nitro-PAHs, facilitating their transformation into intermediate compounds that can be further degraded into non-toxic byproducts (Deng et al., 2023; Marvin-Sikkema & de Bont, 1994). Bacteria from the genera *Acinetobacter*, *Bacillus*, *Micrococcus*, *Mycobacterium*, *Pseudomonas*, *Rhodococcus*, *Sphingomonas*, and *Xanthomonas* are well-known for their capacity to breakdown PAHs (Mohapatra & Phale, 2021; Sarma et al., 2024). These bacteria use PAHs as a carbon and energy source, which aids in the breakdown of complicated PAH rings. Fungi, such as those from the genera *Cunninghamella*, *Phanerochaete*, *Pleurotus*, and *Trametes* provide substantial contributions (Acevedo et al., 2011; Wójcik et al., 2022). They produce lignin-degrading enzymes such as laccases and peroxidases, which can oxidize nitro-PAHs (Cerniglia, 1997; Cerniglia et al., 1985).

Nitro-PAH degradation takes place in two environments aerobic (oxygen-rich) and anaerobic (oxygen-depleted) environments (Fig. 2.3). These processes have multiple critical stages: enzymatic reduction of nitro groups, ring cleavage, formation of central intermediates, mineralization, and assimilation (Ju & Parales, 2010; Roldán et al., 2008). The first stage in aerobic biodegradation of nitro-PAHs is to reduce the nitro groups into an amino group (NH_2) (Lee et al., 2022). This reduction results in a chemical transition that breaks the nitrogen-oxygen bond and transfers electrons to the nitro group. This process alters the chemical structure of nitro-PAHs, making them less complex and more vulnerable to subsequent degradation by microbial enzymes. Nitroreductases play an important role in the abovementioned reduction process, typically exhibiting selectivity for nitro-PAHs with different substrate specificities (Čėnas et al., 2021; Roldán et al., 2008). Following this reduction, the aromatic ring of nitro-PAHs is hydroxylated, increasing the water solubility of the amino-containing nitro-PAHs and allowing them to dissolve in water (Fu, 1990). Dioxygenases, such as Rieske non-heme ring-hydroxylating oxygenase (RHO) and cytochrome P450 monooxygenases (CYP450s), assist this hydroxylation process (Cheng et al.,

2022; Keenan & Wood, 2006). These intermediate compounds undergo structural rearrangement by dehydrogenase enzymes, which restore the aromaticity of the molecular ring. The resulting dihydroxy aromatic intermediates then act as substrates for intradiol and extradiol dioxygenases. Extradiol dioxygenases cleave the aromatic ring between two hydroxyl groups positioned ortho to each other, while intradiol dioxygenases break the ring at the meta position through an oxygen-mediated process (Lipscomb, 2008). This cleavage yields intermediates such as catechol. Catechol is subsequently transformed into a primary alcohol, which is oxidized to form the respective aldehyde (Pandolfo et al., 2023; Singh et al., 2015). This aldehyde is then transformed into fatty acids, triggering various metabolic processes, including beta-oxidation, which yields acetyl-CoA. Acetyl-CoA subsequently enters the tricarboxylic acid (TCA) cycle and produces CO_2, H_2O, ATP, and NADH.

Anaerobic biodegradation, unlike aerobic biodegradation, frequently occurs in the presence of chemicals such as nitrate (NO_3^-), sulfate (SO_4^{2-}), manganese (IV), iron (Fe^{3+}), CO_2, or methanogen (Fu & Herreno-Saenz, 1999; Mu et al., 2022). The reductive process is highly effective in the anaerobic degradation of nitro-PAHs, as it involves the enzymatic conversion of nitro groups into amino groups. This transformation is primarily driven by nitroreductase enzymes, which are secreted by certain anaerobic bacteria and play a crucial role in catalyzing the reduction reaction (Boddu et al., 2020; Roldán et al., 2008). These enzymes transfer electrons from donors to the nitro group, producing amino-PAHs or hydroxylamine intermediates. Anaerobic microorganisms use multiple metabolic pathways to break down amino-PAHs after the reduction step (Ladino-Orjuela et al., 2016; Sigvardson & Birks, 1984). One typical mechanism is the reduction of the aromatic ring, which produces cyclohexadiene and cyclohexenes by reducing nitro-PAH aromatic rings successively (Mulder et al., 2019; Vogt et al., 2009). This reduction is facilitated by a variety of enzymes, including reductive dehalogenases, which remove halogen substituents from aromatic rings (Łomża et al., 2023; Wang et al., 2023). The resultant cyclohexadiene and cyclohexenes are then processed by various enzymes to produce more specific aromatic or aliphatic compounds. Aside from aromatic ring reduction, anaerobic microorganisms may use various methods to degrade nitro-PAHs. Hydroxylation adds hydroxyl groups to aromatic rings, whereas the methyl group oxidation route removes them from the nitro-PAH structures (Nishioka & Lewtas, 1992; Zeng et al., 2017). These processes involve enzymes such as hydroxylases and oxidoreductases, which are required for the breakdown of nitro-PAHs. Bandowe and Meusel (2017) highlighted

Mycobacterium sp., capable of degrading nitro-PAHs via both oxidative and reductive pathways.

Under aerobic conditions, 1-nitropyrene undergoes oxidation, leading to the formation of 1-nitropyrene-9,10-dihydrodiol and 1-nitropyrene-4,5-dihydrodiol. In contrast, when oxygen is absent, 1-nitropyrene undergoes a reductive transformation, resulting in the production of 1-aminopyrene (Bandowe & Meusel, 2017). Furthermore, Li et al. (2023) revealed that the *Sphingobium* sp. strain JS3065 degrades 1-nitronaphthalene into 1,2-dihydroxynaphthalene, a precursor in the naphthalene degradation pathway (Li et al., 2023). These findings highlighted the enormous potential of utilizing microorganisms to improve the number of substrates suited for successful cleanup, particularly persistent nitroaromatic compounds.

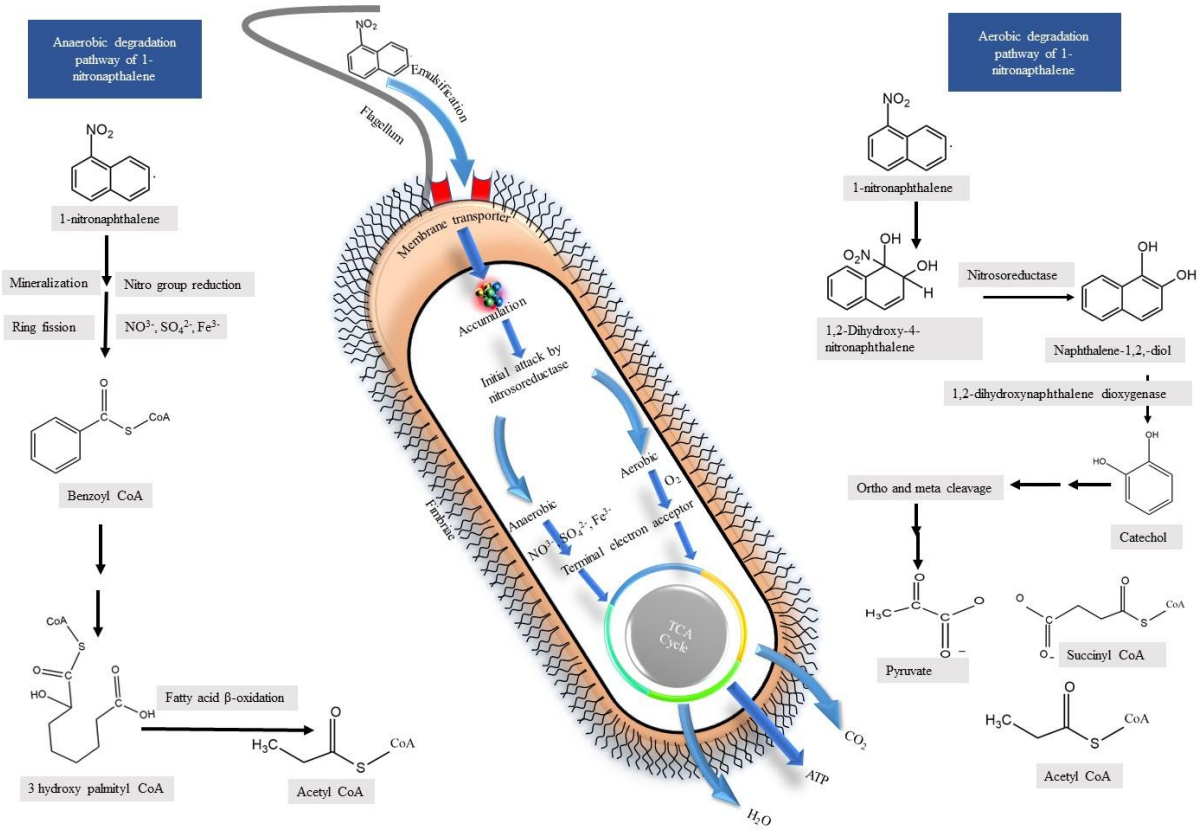


Fig. 2.3. Microbial remediation of nitro-PAHs. Microbes degraded the nitro-PAHs through aerobic and anaerobic environments (Sarma et al., (2024) with permission).

Simultaneously, animals and their gut microbiota have been associated with the breakdown of nitro-PAHs, within the host body via metabolic activities. However, the metabolic breakdown

of nitro-PAHs frequently results in the formation of intermediate molecules. These intermediates can be more hazardous than the original contaminants, posing serious health concerns to the host organism (Garcia et al., 2022; Ning et al., 2020).

Two primary metabolic routes can lead to the early metabolism of nitro-PAHs: either the aromatic ring oxidation or the nitro functional group's reduction (Claus et al., 2016). Oxides, trans-dihydrodiols, tetrahydrotetrols, hydroquinones, quinones, phenolic compounds, benzylic alcohols, and ketones are among the phase I ring-oxidized metabolites produced by aerobic nitro-PAHs metabolism (Moorthy et al., 2015; Von Tungeln & Fu, 1989). Both bacterial and mammalian enzyme systems are capable of reductive nitro-PAHs metabolism in anaerobic or hypoxic environments (Penning et al., 2022).

In mammalian systems, reductive enzymes can be found in their hepatic cytosol and microsomes. Cytochrome P450 and NADPH-cytochrome P450 reductase are found in hepatic microsomes, whereas xanthine oxidase, aldehyde oxidase, and DT-diaphorase are found in the cytosol (Čėnas et al., 2021; Stiborová et al., 2005). Nitro-PAHs are reduced in three steps: the formation of the corresponding nitroso-PAH, the subsequent reduction to the N-hydroxyamino-PAH, and finally the reduction to the amino-PAH. Although each step involves a two-electron mechanism, the reduction mechanism might include one- and two-electron transfers (Kielhorn et al., 2003). Nitroso and N-hydroxyamino metabolites are possible electrophiles, which adds to the metabolic process's complexity.

The degradation mechanisms of these compounds depend on a complex interplay of enzymes encoded by highly conserved nitro-PAH-catabolic genes. Among these enzymes, nitroreductase plays a crucial role in the process and is found in a diverse range of bacteria, including both gram-negative and gram-positive species, as well as symbionts, pathogens, and free-living bacteria (Liu et al., 2018; Roldán et al., 2008). Several nitroreductase-encoding genes have been reported and sequenced, and phylogenetic analysis indicates that these genes are closely related to the *Escherichia coli nfs*A and *nfs*B genes, which encode nitroreductases NfsA (group A) and NfsB (group B) (Roldán et al., 2008; Vass et al., 2009). Similarly, *nit*A and *nit*B encode NitA and NitB, which are closely related to NfsA (Kutty & Bennett, 2005). Similarly, Dioxygenases, which are encoded by genes such as *nid*A, *nid*B, *pah*A, and *pah*B, as well as monooxygenases, notably cytochrome P450 enzymes encoded by *p450* genes, are also important in nitro-PAHs breakdown (Chakraborty et al., 2023; Takizawa et al., 1994). *Rhodococcus*, *Mycobacterium*, and *Nocardioides* have specific catabolic genes, such as *nar*,

phd, *nid*, and *pdo*, that are known for their ability to digest aromatic hydrocarbons (Bengtsson et al., 2013; Patel et al., 2024). Catabolic genes like *nah*, *nag*, *ndo*, *pah*, and *phn* are abundant in *Pseudomonas, Ralstonia, Burkholderia, Sphingomonas*, and *Polaromonas*, all of which can degrade aromatic hydrocarbons (Chikere & Fenibo, 2018; Kaur et al., 2023). *nag* is a broadly conserved nitro-PAH-catabolic gene found in *Sphingobium* sp. and *Ralstonia* sp. (Li et al., 2023; Sazonova et al., 2023). It contributes to the degradation of 1-nitronaphthalene to 1,2-dihydroxynaphthalene. Another highly conserved nitro-PAH-catabolic gene, *nah*Ac, can be found in a variety of gram-negative bacterial species (Lu et al., 2019; Mawad et al., 2020).

2.7. Factors Affecting Phytoremediation and Microbial Remediation Efficiency

Phytoremediation and microbial remediation are two potential bioremediation technologies for addressing the adverse effects of a wide range of environmental contaminants, including nitro-PAHs. The efficiency of these remediation methods can be notably impacted by a myriad of factors that come into play during the degradation processes. Both abiotic and biotic factors play a crucial role in shaping the aforementioned remediation method (Sunday & Joan, 2024; Moxley et al., 2019). Among the abiotic factors, temperature stands out as one of the most influential elements affecting the remediation mechanisms. The role of temperature is paramount in determining the metabolic activity of both plants and microbes (Alkorta et al., 2017; Khan et al., 2013). Elevated temperatures have the effect of hastening enzymatic reactions and microbial metabolism, consequently leading to an accelerated rate of phytoremediation (Magdziak et al., 2015). Conversely, lower temperatures have the opposite effect, slowing down these crucial processes. For example, microbial communities thriving in warmer climates may demonstrate heightened degradation performances at increased temperatures, whereas those inhabiting colder regions might exhibit lower efficiency levels (Abatenh et al., 2018; Zhu et al., 2024). Consequently, the optimization of temperature settings emerges as a critical factor in maximizing the degradation efficiency across diverse environmental conditions. Furthermore, pH is another critical factor that influences the activity of microbial and plant enzymes involved in the breakdown of nitro-PAHs (Gabriele et al., 2021; Tiwari et al., 2019). Any deviations from these optimal pH conditions can have detrimental effects on enzyme functions and microbial proliferation, consequently diminishing the overall efficiency of nitro-PAH degradation processes. Moreover, the availability of substrates, which pertain to the accessibility and concentration of contaminants in the environment, directly

influences the efficiency of their breakdown (Afegbua & Batty, 2018). An adequate concentration of substrates is imperative for sustaining microbial growth and metabolic functions. Higher concentrations of nitro-PAHs serve as a substantial carbon source, fostering increased microbial growth and enhanced degradation capabilities. Nonetheless, excessively high concentrations may overwhelm microbial populations, surpassing their tolerance thresholds and impeding degradation activities (Gogoi et al., 2025). Elevated concentrations of pollutants can also negatively impact the health of plants (Reddy et al., 2020). The availability of key nutrients such as nitrogen, phosphorus, and trace elements plays an important role in the microbial and plant-mediated degradation of organic pollutants (Arulazhagan & Vasudevan, 2011; Wang et al.,2022). These nutrients are essential for plant and microbial development, as well as enzyme synthesis. Deficiencies of these nutrients can hinder the activities of plants and microbes, thereby reducing the overall efficiency of the degradation processes. The composition of the microbial community present in the environment holds significant importance in the degradation of nitro-PAHs. Different microbial strains exhibit varying capacities for breaking down pollutants. A diverse microbial consortium comprising multiple species with complementary metabolic pathways can bolster the resilience and efficiency of the degradation processes (Li et al., 2021; Zhang & Zhang, 2022). This diversity offers a form of backup, ensuring that even if certain strains are hindered by unfavorable conditions, other strains can continue the degradation activities. Research studies have indicated that microbial consortia tend to be more effective in degrading complex pollutants when compared to single microbial strains (Lü et al., 2024; Lv et al., 2025).

Similarly, selecting suitable plant species is another crucial regulatory factor of phytoremediation initiatives (Kafle et al., 2022). Plants have various capacities for absorbing, translocating, and metabolizing contaminants. The efficiency of phytoremediation is determined by the plant's capacity to store and transport pollutants, as well as its growth rate and tolerance to toxins. Selecting the suitable plant species can considerably improve the efficacy of phytoremediation efforts.

2.8. Key Factors Influencing the Efficiency of Bioremediation Technologies for nitro-PAHs Degradation

Biostimulants are biological compounds including plant growth-promoting rhizobacteria, humic substances, mycorrhizal fungi, and seaweed extracts that play an important role in improving plant health and growth through various mechanisms such as increased nutrient

availability, improved root development, and increased resistance to environmental stresses such as salinity, drought, pH, and pollutant toxicity (Bhupenchandra et al., 2022; Kumari et al., 2022) (Fig. 2.4). The utilization of these compounds in the process of phytoremediation is crucial as they contribute significantly to improving its effectiveness. Notably, humic compounds, a prominent class of biostimulants, have been reported to significantly enhance plant absorption of macronutrients and trace elements (Bernstein et al., 2019; Savarese et al., 2022). They increase proton-ATPase activity in root cell plasma membranes (Zandonadi et al., 2010). Proton-ATPase is an enzyme that hydrolysis the ATP to create an electrochemical gradient, which facilitates the absorption of nitrate ions and other nutrients into plant roots, thereby improving nutrient uptake, plant growth, and overall health (Haruta et al., 2015; Ku et al., 2022). Several studies have reported that humic acids enhance the phytoremediation efficiency of various plant species. For example, Sung et al. (2013) reported that the application of humic acid boosted the petroleum hydrocarbon remediation efficacy of *Phragmites communis* (Sung et al., 2013). Similarly, Zhang et al. (2023) reported that humic acid improved the phytoremediation effectiveness of *Typha orientalis*, *Tagetes patula*, and *Menthe aquatica* (Zhang et al., 2023). Seaweed extracts are another important class of biostimulants known for their positive effects on phytoremediation processes (Khattabi et al., 2023). These extracts, rich in hormones and nutrients, provide significant support for plant development and resistance to environmental challenges. Notably, the brown seaweed *Ascophyllum nodosum* has been reported to effectively degrade PAHs in soil (Boudh et al., 2019). Similarly, extracts obtained from the green seaweed *Ulva lactuca* can remediate PAHs from polluted water bodies been shown to be capable of breaking down PAHs found in contaminated water bodies, demonstrating their potential in the realm of aquatic phytoremediation (Areco et al., 2021).

In the realm of microbial remediation, biostimulants play a crucial role in augmenting the activity and proliferation of beneficial soil microorganisms present in the contaminated sites. These biostimulants provide necessary nutrients, and growth ingredients, that efficiently promote microbial activity (Chen et al., 2002). Furthermore, biostimulants play a vital role in enhancing the bioavailability of contaminants in the soil, as well as encouraging and maintaining interactions between plants and microbiomes (Ali et al., 2023; Bartucca et al., 2022). The impact of biostimulants extends to enhancing soil aggregation and porosity, thereby creating a conducive environment for the thriving of microbial communities. Overall, the use of biostimulants facilitates the synergistic association between plants and microorganisms, resulting in a large increase in the overall efficiency of the microbial and phytoremediation

process.

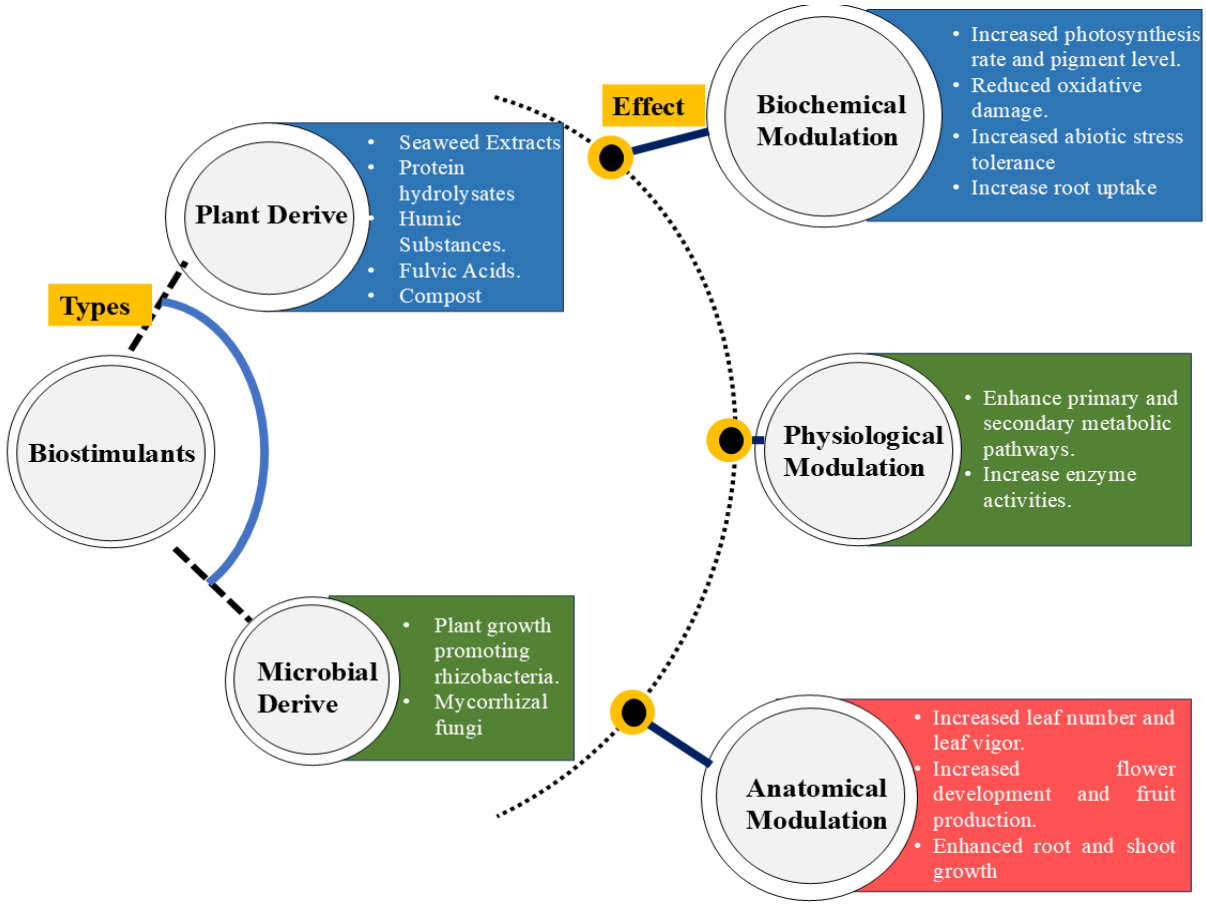


Fig. 2.4. Role of biostimulants in physiological modulation, anatomical modulation, and biochemical modulation. Biostimulants positively affect plants through three key mechanisms: biochemical modulation, physiological modulation, and anatomical modulation. Biochemical modulation improves photosynthesis efficiency, reduces oxidative stress, enhances abiotic stress tolerance, etc. Physiological modulation stimulates primary and secondary metabolic pathways. Anatomical modulation leads to visible structural improvements, such as increased leaf number and vigor, enhanced flower and fruit production, and better root and shoot growth. Collectively, these effects contribute to overall plant health, and stress resilience.

2.9. Integrated Bioremediation: Enhancing Efficiency Through Synergistic Approaches

The effectiveness of individual remediation strategies, such as phytoremediation and microbial remediation, is significantly influenced by key regulatory factors, including temperature, pH, substrate availability, nutrient concentrations, plant species, and microbial community composition. Given the inherent limitations and cost-benefit considerations of plant- or

microbe-based remediation methods, optimizing their performance requires a strategic combination of complementary approaches. Consequently, integrated remediation techniques will become the most efficient method for the removal of nitro-PAHs from contaminated soils. Specifically, the incorporation of biostimulants and microbial inoculants into phytoremediation frameworks presents a technologically viable, cost-effective, and environmentally sustainable approach to mitigating nitro-PAH contamination.

In microbe-enhanced phytoremediation, root exudates containing organic acids serve as chemical signals that attract beneficial microbes into the rhizosphere (Chen & Liu, 2024; Upadhyay et al., 2022). Additionally, the introduction of microbial inoculants further enhances the overall effectiveness of the remediation process. These exudates that are released by the plants contain sugars, amino acids, and organic acids, which play an important role in the growth and development of microorganisms (Canarini et al., 2019). Similarly, bacteria produce biosurfactants that aid in the dispersion of hydrophobic compounds such as nitro-PAHs. This process enhances the bioavailability of nitro-PAHs, making them easier for plants and other microbes to uptake and metabolize. Simultaneously, the application of biostimulants increases the activity of proton-ATPase in root cell plasma membranes, increasing the plant's ability to absorb nutrients. Furthermore, biostimulants promote root growth and exudate release, hence increasing microbial colonization and activity in the rhizosphere. Plants use some mechanisms such as phytovolatilization, phytoextraction, and phytostabilization to breakdown pollutants, whereas microorganisms metabolize PAHs via several metabolic pathways (Khan et al., 2022; Nedjimi, 2021). These pathways involve multiple transformations, including nitro group reduction, hydroxylation, and ring oxidation. These reactions eventually lead to the formation of Krebs cycle intermediates, which are further broken down into carbon dioxide, water, and ATP as a source of energy. The incorporation of biostimulants and microbes in phytoremediation resulted in a powerful approach to combat environmental contamination, particularly in the case of hazardous persistent pollutants such as nitro-PAHs. This comprehensive strategy improved the efficiency of pollution removal while also enhancing the well-being of the surrounding ecosystem, establishing it as a beneficial approach for long-term environmental management.

2.10. Conclusion and Future Prospects

Nitro-PAHs are hazardous, widespread environmental pollutants and pose serious threats to the environment, and human health. Their persistence in soil, water, and air, mostly due to

industrial activities, vehicular emissions, and incomplete combustion of organic matter, makes them a serious concern. Given their structural similarity to parent PAHs, nitro-PAH derivatives exhibit comparable toxicological effects, even heightened carcinogenic and mutagenic potential. The urgent need for effective remediation strategies has led to a growing interest in bioremediation, which has emerged as a promising, sustainable approach. However, several obstacles remain in optimizing its efficiency and scalability. The complex structure of nitro-PAHs necessitates the selection of specific plants and microbial strains with specialized enzymatic pathways capable of rapid degradation. Screening and identifying these microbes are a difficult endeavor due to the varied microbial populations seen in polluted settings. To overcome these limitations, further study into microbial diversity, metabolic pathways, and environmental interactions is needed to improve degrading efficiency. Moreover, co-contaminants including heavy metals, PAHs, and other persistent organic pollutants may interfere with microbial activity, and hinder the degradation process. Developing techniques to overcome these effects and strengthen microbial resilience will be critical for maximizing bioremediation results. The incorporation of biostimulants into the bioremediation also enhanced the degradation of organic pollutants by supplying vital nutrients and boosting microbial activity. However, selecting appropriate biostimulants is crucial to ensure they supply the necessary carbon sources, electron acceptors, and donors to stimulate targeted metabolic pathways. Determining optimal biostimulant application rates is equally important. Furthermore, monitoring the environmental fate of biostimulant residues is critical for determining their long-term ecological effects and preventing secondary contamination. Advances in monitoring techniques, particularly real-time evaluations of microbial activity and degradation kinetics, can help us better understand bioremediation dynamics and optimize processes. Additionally, emerging technologies such as nanoparticle-based delivery systems and biochar amendments present promising solutions for increasing biostimulant efficiency and microbial survival in contaminated environments. Fostering collaborations with environmental agencies, legislators, and agricultural stakeholders is critical for incorporating microbial-assisted bioremediation into sustainable land management techniques. Furthermore, continuous monitoring, cost-benefit analysis, and environmental impact studies will be required to ensure widespread acceptance and regulatory approval of these technologies. By resolving these obstacles, bioremediation can emerge as a feasible and ecologically sustainable solution to nitro-PAH contamination, helping to restore ecosystems and preserve public health.