Review
43

Egypt. J. Bot., **Vol. 62,** No.3, pp. 611-640 (2022)

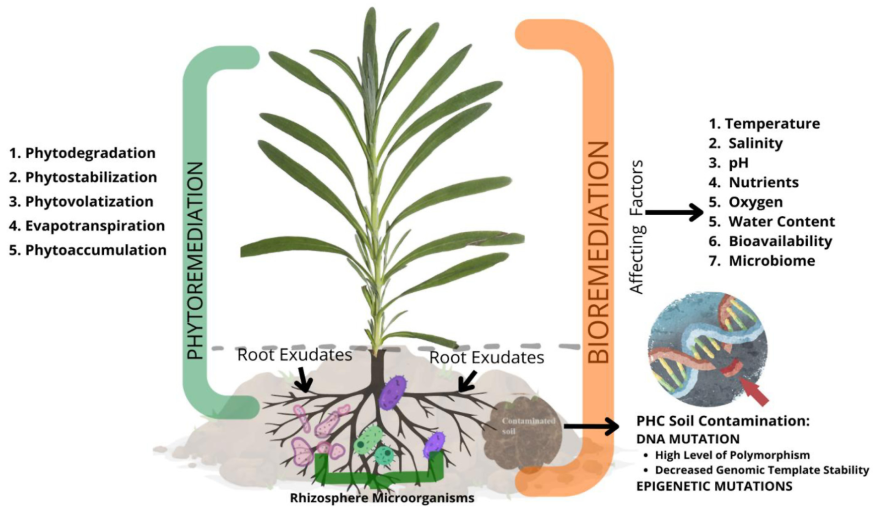
Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Phytoremediation of Crude Petroleum Oil Pollution: A Review

Zahra S. Hussein[(1)#], Nashwa Hamido[(1)], Ahmad K. Hegazy[(2)#], Mohamed A. El-Dessouky[(3)], Nermen H. Mohamed[(4)], Gehan Safwat[(1)]

[(1)]Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA), 6th of October, Giza 12451, Egypt; [(2)]Department of Botany and Microbiology, Faculty of Science, Cairo University, Giza 12613, Egypt; [(3)]Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt; [(4)]Egyptian Petroleum Research Institute, Nasr City, Cairo 11727, Egypt.

Graphical Abstract



ENVIRONMENTAL pollution is exacerbated by the rise in petroleum hydrocarbons due to exploration, production, transportation, and industrialization. This requires immediate remediation. Although crude oil removal using conventional techniques is efficient for cleaning up aquatic and terrestrial ecosystems, it is costly and requires specialized staff and equipment. Despite their negative environmental consequences, chemical compounds such as dispersants, cleansers, emulsifiers, biosurfactants, and soil oxidizers are highly utilized. Phytoremediation and bioremediation have emerged as cost-effective and environmentally friendly technologies. This paper aims to review the impacts of crude oil pollution and the phytoremediation of polluted ecosystems. We have reviewed various phytoremediation/bioremediation mechanisms and environmental factors. Additionally, we have discussed the degradation of crude petroleum, factors affecting petroleum hydrocarbon bioremediation, and the environmental consequences, such as DNA and epigenetic mutations. We have also compared the economics of phytoremediation and restoration of polluted sites with conventional technology. Plants can remediate the environment through phytodegradation, phytostabilization, phytovolatilization, evapotranspiration, and phytoaccumulation. The microbial activities in the plant rhizosphere enhance the degradation and accumulation of the pollutants and modulate their bioavailability, thereby remediating the polluted areas and stabilizing the soil fertility.

Keywords: Ecological impacts, Economics of phytoremediation, Functional traits, Future perspectives, Genetically modified plants, Mechanisms of phytoremediation.

#Corresponding authors emails: zsaleh@msa.edu.eg Phone: +201012422213; hegazy@sci.cu.edu.eg Phone: +201222430272
Received 19 /05/2022; Accepted 11/08/2022
DOI: 10.21608/ejbo.2022.136551.1980
Edited by: Prof. Dr. Salama A. Ouf, Faculty of Science, Cairo University, Giza 12613, Egypt.

Introduction

Soil pollution caused by human activities has critically threatened ecosystems and human health (Naeem & Qazi, 2020). Currently, several global locations are polluted with organic and inorganic compounds of petroleum hydrocarbons (PHC) (Gao et al., 2022), which are refined crude oil products. During exploration, transformation, and industrialization of oil, crude oil is released and spread into the surrounding environment via chemical (auto-oxidation and photooxidation), physical (dispersion), biological (hydrocarbons bioremediation and phytoremediation), and physiochemical (sorption, dissolution, and evaporation) interactions (Truskewycz et al., 2019). The oil transformation processes include altering the exposure, composition, execution, and toxicity level of extracted petroleum. PHC pollutants are a significant risk for living organisms due to their toxicity, carcinogenicity, mutagenicity, and teratogenicity (Yap et al., 2021). Chang et al. suggested that direct ingestion or food chain cycling (soil-plant-human or soil-plant-animal-human), consuming contaminated groundwater, and reduced land usability from PHC-polluted soil put humans and ecosystems at significant risk, as shown in Fig. 1. The US Environmental Protection Agency has classified most crude oil components as priority pollutants (USEPA, 2009). PHC-contaminated soil can adversely impact human health and cause serious diseases such as cancer and other immune system and reproductive system disorders (Varjani & Gnansounou, 2017; Suganthi et al., 2018).

Systematic research programs, including assessment and remediation, are required to protect and restore polluted ecosystems. Recent efforts have been directed towards directly reducing the pollution levels on-site and preparing appropriate environmental guidelines for polluted site remediation (Gaur et al., 2021). Several biological, biochemical, and physical techniques, including in-situ and ex-situ treatments, have been developed for the remediation of crude oil-contaminated ecosystems (Uzoije & Agunwamba, 2011). The limitations of these technologies include the cost, their application at high pollution levels and in mixed organic and inorganic pollutants, and the irreversible changes in physicochemical properties of ecosystems (Kumari et al., 2012; Marchand et al., 2014; Hoang et al., 2021). The current review focuses on the consequences of crude petroleum oil pollution on ecosystems and the remediation of contaminated ecosystems using bioremediation, particularly phytoremediation, as a cost-effective technique.

Ecological impacts of oil pollution

The effect of crude oil pollution on the environment depends on the characteristics of the ecosystem and the prevailing environmental characteristics (Lopes et al., 2009). Crude oil pollution can directly contaminate terrestrial ecosystems, while the dispersed oil that floats and spreads on the water surface affects both the terrestrial and aquatic environments (Hegazy, 1997; Pezeshki et al., 2000). The presence of crude oil causes several changes, including increased organic matter due to the death and decomposition of affected organisms, altered soil pH, and accumulated heavy metals (Ekundayo & Obuekwe, 2000). These alterations and species' differentiated sensitivity result in modifications in the community's composition (Lopes et al., 2009).

Oil pollution might affect living organisms in different ways, including uptake through the food chain, absorption, inhalation or ingestion of harmful oil components, and direct dermal contact (Buskey et al., 2016). The resulting toxicity can be either chronic or acute. Chronic toxicity is defined as the effects resulting from continuous and long-term exposure to oil pollution. In contrast, acute toxicity is caused due to shortterm exposure to high oil concentrations (Chang et al., 2014). The duration of exposure, the concentration of released oil, hydrocarbon resistance, availability, and attention and bioaccumulation of other oil components determine whether the toxicity is acute or chronic. Of these two types, chronic toxicity might affect living organisms at various levels of the organization and cause alterations in the biological organization of ecosystems (Hegazy, 1995; Rekadwad & Khobragade, 2015).

Impact on aquatic ecosystems

Each ecosystem is unique; hence, the effects of PHC on these have distinct parameters. Aquatic ecosystems are complex in structure due to the variations of the interacting biological diversity and food webs. Crude oil pollution has different impacts on the ecosystem depending on its structure and function. Also, PHC contaminant deposition in aquatic ecosystems threatens biodiversity (Perhar & Arhonditsis, 2014; Chibuzor Nwadibe et al., 2020). The effects of PHC pollution on water cause ecological imbalance and negatively impact microorganisms, plants, animals, and humans (Rodrigues et al., 2010; Laffon et al., 2016;

Sarria-Villa et al., 2016; Mostafa et al., 2021). When aquatic organisms are exposed to oil spills, hydrocarbons can accumulate in their tissues and cause bioaccumulation, which is transferred to humans via the food chain (Laffon et al., 2016). The chemical composition and exposure level of the polluted oil mainly determine the response of biological diversity to oil pollution. Certain biological traits make some species more vulnerable to oil contamination than others (Chang et al., 2014).

As crude oil usually floats on the surface of the water, species residing on the water surface, such as kelp and seagrass species, are more affected than the deep-water biodiversity species. Species, such as marine mammals and birds in regular contact with the air-water interface are more vulnerable to oil exposure (King et al., 2021). Toxicity pathways in marine species include ingestion of oil, accumulation of pollutants in the organism's tissues, DNA damage, immune and cardiac dysfunction, and mass mortality of eggs and larvae. Species respond differently to oil pollution due to their morphological, genetic, and physiological variations. The species' response to crude oil pollution has been predicted based on previous research on genetically related species (Leighton, 1993; Rekadwad & Khobragade, 2015; Buskey et al., 2016; Langangen et al., 2017; Grosell & Pasparakis, 2021).

Reports have shown the lethal and sublethal effects of PHC on fish, including physical deformity, abnormal neuronal development, DNA damage, and changes in biological activities such as migration, feeding, and reproduction (Langangen et al., 2017; Osuagwu & Olaifa, 2018; Meador & Nahrgang, 2019; Grosell & Pasparakis, 2021). Oil pollution has numerous consequences on seabirds, including pathological changes in the lungs, kidneys, liver, salt glands, and intestinal tract that might cause immunotoxicity, hemolytic anemia, dysfunction, reproductive toxicity, and endocrine disruption (Leighton, 1993; Perhar & Arhonditsis, 2014; King et al., 2021). Turtles, shellfish, and some coastal vertebrate species are also negatively affected by PHC pollution (Perhar & Arhonditsis, 2014).

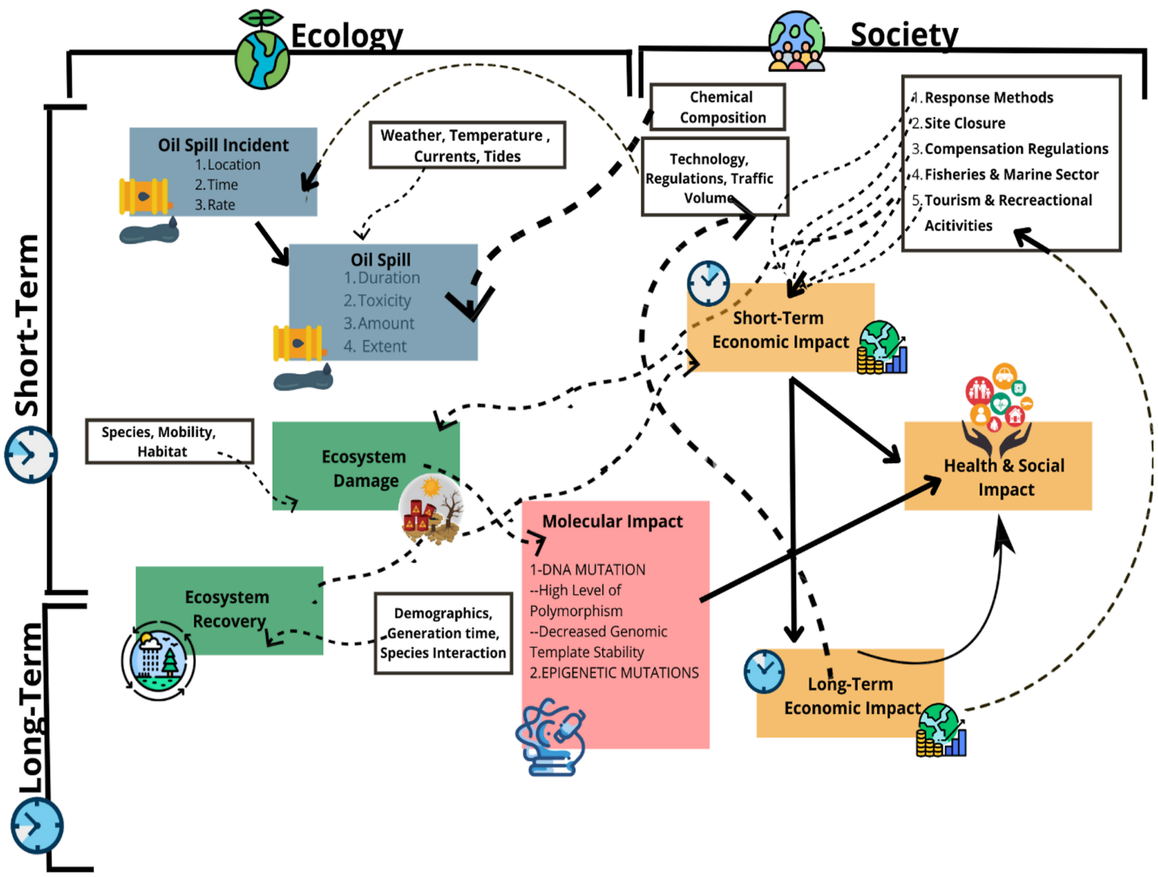


Fig. 1. Crude petroleum oil spill impacts

Exposure to PHCs causes physical and chemical damage to terrestrial or aquatic plants. The impact of the oil depends on the characteristics and prevailing environmental conditions of the oil. Low levels of oil pollutants can be predicted by assessing plant tissue toxicity. Heavy oil pollutants cause asphyxia and alter gaseous exchange since they cover the plants (Pezeshki et al., 2000). Arellano et al. (2017) and Truskewycz et al. (2019) reported that crude oil pollution affects temperature regulation and reduces photosynthesis, causing excessive shedding of leaves. It also coats the roots, hence disrupting nutrient and water uptake.

Mostafa et al. (2021) reported that aquatic plants' functional and structural traits like *Azolla pinnata* are altered after exposure to crude oil. The affected plants show decreased total chlorophyll content and altered amounts of carotenoid content. Crude oil treatment also affected the plant's genetic material and significantly damaged the plant's tissues and chloroplast structure. Atta et al. (2020) showed a similar effect of crude oil on photosynthetic pigments of *Eichhornia crassipes,* including alterations in the chlorophyll and carotenoids. Lopes et al. (2009) showed that crude oil pollution adversely affected the aquatic flora and dynamics of flood plains. This study examined the effects of crude oil on two Amazonian plants, *Eichhornia crassipes* and *Echinochloa polystachya*, showing an increase in plant mortality at high pollution levels. However, *E. crassipes* showed higher sensitivity to crude oil pollution due to anatomical alterations such as inhibited leaf and root growth, and reduced leaf numbers.

Impact on terrestrial ecosystems

Soil is a major terrestrial natural resource and is the material basis for social development and sustainable economics. Wang et al. (2017) reported the harmful consequences of crude oil-mediated soil pollution, including adverse effects on (1) Physical and chemical properties of soil, (2) The microbial community, (3) Plant growth and functional traits, and (4) Human and animal health. The state of environmental pollution can be investigated using the hydrocarbon pollution level as a steady indicator (Mueller & Shann, 2006).

PHCs mainly affect the chemical characteristics of soil by causing a phenomenal increase in micronutrient elements (Mn, Cu, Fe, and Zn), total petroleum hydrocarbons (TPH), and cadmium (Cd) and lead (Pb) content. Crude oil pollution lowers the pH of the affected soil than unpolluted sites. The carbon (C) and nitrogen (N) content of petroleum oil significantly increases the total organic carbon (C) and nitrogen (N) in oil-polluted soil compared with normal soil. Additionally, oil pollution reduces the soil phosphorus (P) content and the effective cation exchange capacity (ECEC) and hence, base saturation (Udoh & Chukwu, 2014; Ahmadi et al., 2021). Reports have also shown that oil pollution increases the soil's organic matter while decreasing the exchange of acidity and available phosphorus. Additionally, reports showed a significant increase in the content of total petroleum hydrocarbons and extractable heavy metal levels. Uquetan et al. (2017) illustrated that soil pollution with crude oil destroys the soil structure, increases bulk density, and reduces soil porosity, aeration, soil capillarity, and nutrient availability and uptake by plants.

Crude petroleum alters the physical properties of soil because of its low emulsifying ability, low density, and high viscosity. These facilitate absorption into the soil surface and change the soil porosity and permeability (Wang et al., 2017). Elevated crude oil levels can reduce water and oxygen penetration into the soil and clog the pores. Oil contamination creates anaerobic conditions that affect the soil microbial community by smothering the soil particles and blocking air diffusion. Petroleum oil can reduce saturated hydraulic conductivity and increases the bulk density. Other properties of oil-polluted soil include the formation of water-resistant aggregates and higher hydrophobicity than unpolluted soil. Soil permeability, optimum water content, and maximum dry intensity are also decreased due to petroleum oil pollution (Khamehchiyan et al., 2007; Abosede, 2013; Klamerus-Iwan et al., 2015).

Soil pollution with lubricating and crude oil reduces aeration and compaction, causing poor wettability and increasing heavy metal accumulation propensity. Differential growth retardation occurs due to poor aeration, impaired photosynthesis and transpiration, and soil qualities for root penetration (Khamehchiyan et al., 2007; Uquetan et al., 2017; Bakina et al., 2022). Moreover, oil pollution significantly affects soil properties and crop growth, resulting in the soil being temporarily unsuitable for crops before restoration. PHC increases soil's total nitrogen (N), organic carbon (C), and total hydrocarbons. Additionally, the exchangeable Na^+, K^+, Ca^{2+}, and available phosphorus (P) are increased in PHC-polluted soils. The heavy metal content, including Mn, Pb, and Fe, is also increased (Uzoije & Agunwamba, 2011; Abosede, 2013; Klamerus-Iwan et al., 2015).

Crude oil pollution might stimulate a rise in stress-resistant microbes that can adapt to the polluted environment. Alterations occur in the microbial community's composition and structure and the soil's enzyme system to enable oil degradation. The adapted microorganisms become predominant with an oil-degrading enzyme system (Alisi et al., 2009; Uzoije & Agunwamba, 2011; Moubasher et al., 2015). In the ornamental plant, *Hylotelephium spectabile*, the presence of PHC pollutants in the soil can stimulate the development of specific PHC degraders, including *Actinobacteria*, *Proteobacteria,* and *Acidobacteria*, which significantly increase the relative abundance of PHC degrading genes with an increase in PHC concentration. The plant roots, along with the stimulated degraders, promote the expression of PHC degrading genes and comprise a beneficial-biodegradation community structure for PHC removal from the rhizosphere (Cheng et al., 2019).

Impact on plant structural and functional traits

Studies evaluating the effects of crude oil toxicity on plant anatomy, physiology, and growth parameters showed that crude oil could alter soil properties and plant growth (Skrypnik et al., 2021). The presence of crude oil in the soil adversely affects the chemical, physical, and microbiological characteristics of the soil , reducing plant seed germination and affecting plant growth (Kayode et al., 2009). Similar observations were reported by Uquetan et al. (2017), who described that in control plants, seed germination started after ten days, but in plants exposed to soil polluted with higher doses of crude oil, it started after 16 days. When the oil's volatile fractions enter the seed coat, they reduce seed germination.

Olaranont et al. (2021) studied the effect of crude oil on the physiological and anatomical characteristics of *Ipomoea pes-caprae* and reported a reduction in four physiological characteristics; chlorophyll content, leaf width, leaf length, and survival time. The anatomical characteristics of *Ipomoea pes-caprae*, including the thickness of leaf abaxial and adaxial cutin and leaf blades, and the height of the leaf's spongy layer, were also impacted by the contamination.

Xun et al. (2015) reported that in *Avena sativa*, PHC pollutants inhibited plant growth, subsequently increasing proline and malondialdehyde (MDA) accumulation. The antioxidant activities of the enzymes such as peroxidase, catalase, and superoxide dismutase were also decreased due to petroleum stress. Tesar et al. (2002) reported that petroleum pollution reduces the ryegrass biomass by 96% after 30 days of growth in soil treated with 2.5% oil by mass. Oil pollution negatively affected the seed germination rate and percentage in *Vigna unguiculata*. It also affected growth parameters such as the plant height, leaf number and area, stem girth, and plant leaf development. *Vigna unguiculata* biomass and chlorophyll content decreased while the heavy metal levels in the fruits were elevated, altering their nutritional composition (Jamali et al., 2021).

After a six-month plantation period, crude oil in the soil used to cultivate Cyperus brevifolius significantly reduced the chlorophyll content in the leaves compared with plants raised in unpolluted soil. The content of chlorophyll a, chlorophyll b, and total chlorophyll decreased with increased pollution levels. Crude oil negatively affected the biomass of plant shoots and roots. Increased morphological and anatomical changes such as structural deformations were observed with increased pollution levels (Baruah et al., 2014).

Decreased levels of chlorophyll a, chlorophyll b, and total chlorophyll were seen in *Azolla pinnata* plants grown in oil-polluted soil with the increase in pollution level after different experimental periods (7, 14, and 21 days). The functional traits of *A. pinnata* after a 21-day pollution exposure period were altered, showing severe damage in the structure of chloroplast and frond tissues. This damage includes malformed epidermis, less compacted palisade, disintegrated parenchymal tissues, along with malformation and lysis of chloroplasts (Mostafa et al., 2021).

Different molecular techniques were applied to evaluate the molecular response of plants underlying the toxic effect of crude oil pollution on plants. Understanding the molecular mechanisms of plant response to crude oil stress is essential to improving phytoremediation strategies for removing PHC from polluted soil.

Currently, studies including gene expression analysis of plants grown under crude oil stress are being applied to understand the molecular mechanisms involved in plant tolerance to hydrocarbons. The transcriptome profiles of *Zea mays* grown under varying levels of petroleum oil showed 883, 1281, and 2162 differentially expressed genes in the control (0%) vs. 1% crude oil, 1% vs. 5% crude oil, and control vs. 5% crude oil groups, respectively. The differentially suppressed or induced genes were related to functions, including the response mechanism to osmotic stress and the plant's phytoremediation capability. The differentially expressed genes in *Zea mays* exposed to crude oil pollution included upregulated genes for certain antioxidant enzymes, hormone signaling networks, Versicolorin reductase, and cell wall-related genes. The Derlin general secretion pathway genes were also measured as differentially expressed genes in response to hydrocarbon stress. The expression of Cys protease increased with an increase in crude oil levels. Genes such as Pao, GS2, Enod93, CIPK, and Opr are crude oil stress-responsive genes in *Zea mays* (Cevher-Keskin et al., 2018).

The analysis of mRNA expression profiles in bermudagrass roots raised under PHC-stress showed a complex and multifactorial molecular response. Under normal growth conditions, the plant is sufficiently in contact with the soil and has a root system that allows adequate supply of water and oxygen. Alternatively, the nature of the crude oil-contaminated environment causes drought and anoxic stress along with disruptive chemical stress. Plants sense stress signals, which are communicated by the signaling enzymes. These enzymes regulate the transcription and translation processes to favor the synthesis of enzymes involved in alleviating the harmful effects of crude oil pollution. Based on the complex and multifactorial molecular responses seen in the mRNA expression profiles of plants grown under petroleum stress, the most evident mechanism are anoxic and mechanical stress (Peña-Castro et al., 2006).

A study showed that *phytoene synthase* (PSY) expression is induced in *Salicornia iranica* grown under mild crude oil pollution conditions. Abdollahzadeh et al. (2019) concluded that the PSY gene might activate carotenoids as abscisic acid (ABA) precursors. PSY promotor analysis revealed the presence of responsive cis-acting elements to ABA, suggesting that the PSY gene is involved in abiotic stress response.

PCR-based molecular markers are used to detect the genetic variations caused by crude oil stress by comparing generated profiles of plants raised under polluted and unpolluted environments. SCoT and ISSR molecular markers revealed discriminative profiles in *Vinca rosea* plants, which were grown under varying oil pollutant levels compared with those raised in an unpolluted environment. The band patterns of both markers showed elevated levels of polymorphism along with dose-dependent alterations in DNA profiles with a decreased genomic template stability (GTS) as the petroleum pollution level increases (Hussein et al., 2022). However, while molecular markers are widely used to evaluate the effects of other environmental contaminants these markers have not been adequately utilized to assess the genotoxic effects of PHC contaminants on plants (Neeratanaphan et al., 2014; Ozyigit et al., 2021).

Recently, comet assay or single-cell gel electrophoresis (SCGE) has been widely used to assess genotoxicity. The comet assay indicates variations in the percentage of DNA damage in *Azolla pinnata* plants raised under different levels of oil pollutants. The reduction in DNA damage occurs when exopolysaccharides act as antioxidant compounds and repair the damage in molecules, including DNA (Mostafa et al., 2021). This reduction has been found to decrease with an increase in oil pollution levels. The comet assay was also used to detect the effects of hydrocarbons on organisms, including earthworms, fishes, and humans (Hoshina & Marin-Morales, 2010; Khisroon et al., 2015; Ramadass et al., 2016). This assay is a cheap, fast, and sensitive technique that detects minor DNA damage and is ideal for detecting genotoxicity (Rigonato et al., 2005). We recommend this technique to detect the genotoxicity of plants raised under oil pollution.

The cyto-genotoxic effect of crude oil has also been monitored based on the decrease or increase of the mitotic index (MI). Previous studies showing the cytotoxic effect of PHCs on plants indicated that it reduces the MI of the plants raised under crude oil pollution. Other techniques to assess the cyto-genotoxic impact of oil pollution include determining micronuclei frequency (MN) and chromosomal aberrations (CA). Studies by Wali Alwan (2019), Cruz et al. (2019), Aguelmous et al. (2020), and Haq & Kalamdhad (2021) showed that plants raised under crude oil pollution demonstrated increased MN and CA.

Epigenetics can be used to explain the interactions between a species and several factors such as the chemicals it is exposed to, abiotic environmental components, or another species in a community. Technological progress in the field of epigenetics has provided insights into the epigenetic features such as DNA methylation in certain ecologically relevant species such as plants, algae, fish, and several invertebrates.

Epigenetics can also be used to investigate the inheritable changes that occur with gene expression without altering the DNA sequence. Environmental pollutants such as PHC can induce epigenetic changes in cladocerans, insects, and plants. Although the environmental impact of PHC pollution has been extensively studied, there is still a gap in understanding the molecular mechanism underlying the changes in the composition of the affected plants. In addition to the biological processes, previous studies have shown that epigenetic alterations induced by the natural environment might profoundly affect social and economic development, human health, and lifestyle. Vandegehuchte & Janssen (2014) reported that epigenetic mechanisms such as histone modification and DNA methylation could alter the function of the genome under exogenous influences.

Vandegehuchte & Janssen (2014) and Cañizares-Martínez et al. (2022) showed that oil exposure elicits responses in certain species that are embedded in their genetic structure. This exposure causes immediate alterations in the expression of genes relevant to xenobiotic metabolism and other critical biological processes, leading to improper development. Many responses generate transient changes in function and epigenetic gene regulation, which might continue after extended exposure and result in more irreversible changes. Further, some of these epigenetic modifications are inheritable such as the responses to oil pollution that last for several generations despite never being physically exposed to crude oil pollution. Finally, crude oil pollution might cause mass-mortality events that alter current levels of genomic variation and/or induce selection regimes, favoring individuals with allelic variations that make them more resistant to the detrimental consequences of oil exposure.

Biodegradation and bioremediation of crude petroleum oil

The remediation approaches that have been developed to reclaim crude oil-contaminated soil and water are based on mechanical and physiochemical methods, including electric field application, soil washing, solidification, excavation and landfill, and soil incineration (Sheoran et al., 2010; Wuana & Okieimen, 2011; DalCorso et al., 2019). Nevertheless, these methods have several limitations, including prohibitive cost, inefficiency in sites with low pollution levels, and possible ecosystem deterioration due to irreversible alteration of water and soil's biological and physicochemical characteristics (Ali et al., 2013; DalCorso et al., 2019). Hence, ecofriendly and cost-effective technologies must be adopted for the remediation of crude oil polluted ecosystems.

Due to its high efficiency and low cost, bioremediation is extensively used. Hydrocarbon degradation is mainly processed by the natural microbial populations that act to recover environments polluted with hydrocarbons (Das & Chandran, 2011). Phytoremediation is a technology involving plants to eliminate contaminants or reduce their bioavailability in the ecosystem (Yan et al., 2020). Plants can absorb ionic compounds in the soil through their root systems, even at low concentrations. Plants can remediate contaminated soil and stabilize its fertility using their root systems that extend into the soil. These systems establish a rhizosphere ecosystem (microbiome) for contaminants' accumulation and moderate their bioavailability (Jacob et al., 2018; DalCorso et al., 2019).

Jacob et al. (2018) reported that phytoremediation is a solar-powered autotrophic system with several advantages, including economic feasibility, ease of management, cost-effectiveness, and minimal maintenance. As an eco-friendly technology, it can be implemented at a largescale and reduce the risk of spreading toxic contaminants.

The nature of pollutants and their concentrations influence PHC biodegradation. PHC can be classified into four groups, including saturates, aromatics, resins (amides, sulfoxides, carbazoles, quinolines, and pyridines), and asphaltenes (esters, ketones, fatty acids, phenols, and porphyrins) (Al-Hawash et al., 2018). Some of these compounds might bind chemically to the medium and become difficult to separate. The susceptibility of hydrocarbons to microbial degradation can be generally ranked as (i) Linear alkanes, (ii) Branched alkanes, (iii) Small aromatics, and (iv) Cyclic alkanes, while the high molecular weight polycyclic hydrocarbons (PAH), the variable fractions of hydrocarbons are shown in Fig. 2 (Das & Chandran, 2011; Al-Hawash et al., 2018).

Microbial degradation is the best natural technique for cleaning up PHC-contaminated ecosystems. In 1983, Jones et al. identified petroleum aromatic hydrocarbons biodegradation in marine sediments. Five microorganisms, *Rhodococcus*, *Sphingomonas, Pseudomonas*, *Burkholderia, Mycobacterium*, and *Arthrobacter,* were identified to be able to biodegrade alkyl-aromatics in marine sediments. Adebusoye et al. (2007) reported microbial PHC degradation in a contaminated tropical stream in Lagos, Nigeria. Nine bacterial strains, namely, *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis, Bacillus sp., Alcaligenes sp., Acinetobacter lwoffii, Flavobacterium sp., Micrococcus roseus*, and *Corynebacterium sp*., were isolated from the polluted streams and found to degrade crude oil successfully.

Degradation of complex PHC mixtures in soil and water requires mixed microbial populations with broad enzymatic activities (Das & Chandran, 2011). Das & Mukherjee (2007) found that Acinetobacter can use n-alkanes with chain length C10–C40 as a sole carbon source in petroleum oil-contaminated soil in Northeast India. Generally, fungi, yeast, and bacteria mainly biodegrade the hydrocarbons in the environment. The reported efficiency range of biodegradation is 0.13% to 50% for soil bacteria, 6% to 82% for soil fungi, and 0.003% to 100% for marine bacteria.

Mechanism of soil phytoremediation of oil pollution

Phytoremediation of crude petroleum oil pollution is primarily based on the interaction of plants with contaminated soil. Plants reduce the soil pollution level by several mechanisms, including degradation, volatilization, absorption, and transfer, thus achieving soil remediation (Nedjimi, 2021). Since the 1980s, phytoremediation has become increasingly popular because it is more advantageous than chemical methods (Yan et al., 2020). Phytoremediation can be applied to remove organic contaminants such as PHC in the soil as plants can absorb these contaminants directly and metabolically transform them into different organs (Azab et al., 2016). Also, roots can alter the physical and chemical properties of the soil by secreting exudates that enhance the microbial transformation and degradation of soil pollutants (Suman et al., 2018). The following are the different mechanisms of phytoremediation:

Phytodegradation

Phytotransformation and phytodegradation, which are different terms used for the same process, describe the metabolic capacity of plants. Organic (PAH and TPH) and inorganic (atmospheric nitrogen and sulfur oxides) contaminants are either degraded/ transformed internally via metabolic processes or externally via extracellular enzymes (Das et al., 2010; Kavamura & Esposito, 2010). Phytotransformation processes include root-to-stem and leaf uptake and diffusion for transformation (Cunningham et al., 1995; James & Strand, 2009). The phytodegradation mechanism has been proven to remediate multiple contaminants in soil and water, including petroleum hydrocarbons, pharmaceutical residuals, insecticides, pesticides, and surfactants (Grzegórska et al., 2020).

Although plant-derived enzymes can transform pollutants in soil and sediments, there is little evidence that plants can degrade PHC directly. Enzymes such as dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase that are secreted by plant tissues can catalyze the degradation pathways of pollutants (Nedjimi, 2021).

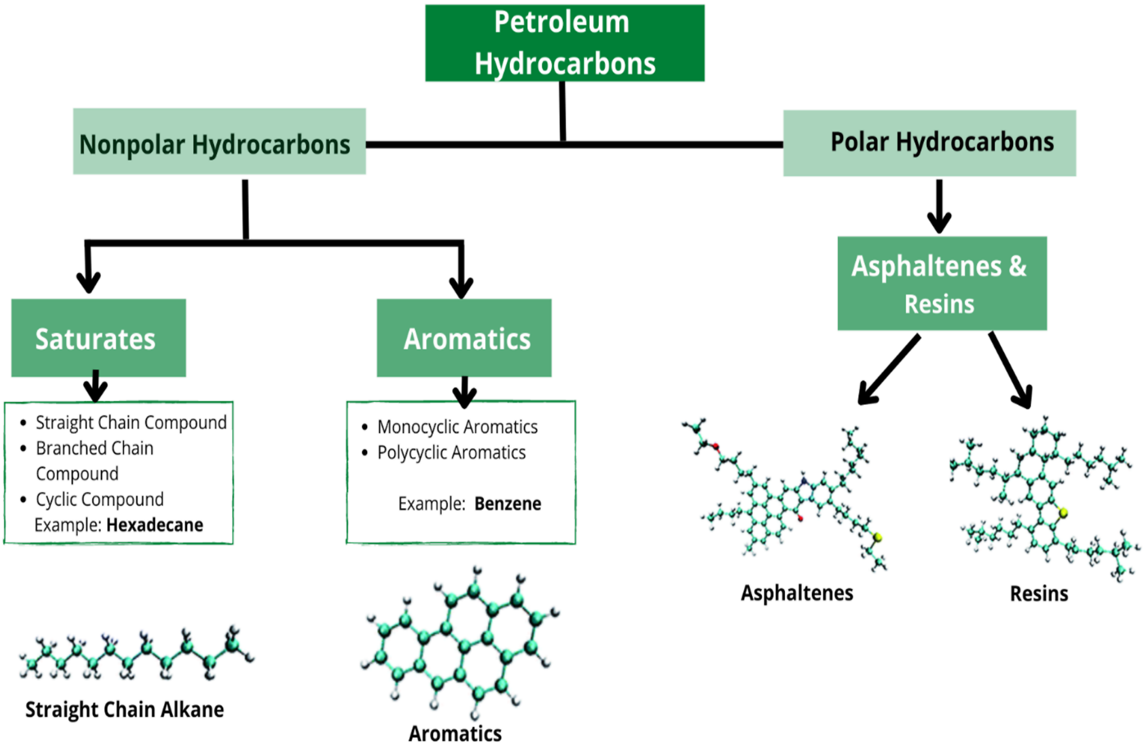


Fig. 2. Variable fractions of petroleum hydrocarbons

Phytostabilization

Adsorption and precipitation are processes used in phytostabilization to concentrate and contain pollutants such as organic compounds and heavy metals found in roots. Rhizospheric processes and soil additives aid in the precipitation and immobilization of soil contaminants, preventing contamination of other ecosystem compartments such as groundwater, bulk soil, and the food chain (Grobelak & Napora, 2015). efficient uptake and accumulation of PHCs by plants through phytostabilization (for example, 2%–8% and 2% accumulation of benzene in alfalfa shoots and roots, respectively) might be used to prevent contaminants from migrating through erosion, leaching, and dispersion. This also helps incorporate organic pollutants into humic materials, which involves binding contaminants to the soil organic matter by plant enzymes or increasing soil organic matter content due to humification. This accounts for the mineralization of four PAH in soils planted with deep-rooted grasses (Germida et al., 2002). Mycorrhizal fungi can sequester heavy metals by chelation and adsorption (Gu et al., 2017). Matanzas et al. (2021) screened a typical brownfield mega-site polluted by Pb and As to identify plants with phytoremediation potential and study the plant-soil interactions. This analysis included a 20-ha study area listing naturally growing plant species. They performed comprehensive habitat classification and generated a unique coverage index in a one-year quadrat study at multiple sampling points. Six herbaceous species, *Dysphania botrys, Lotus corniculatus, Lotus hispidus, Plantago lanceolata, Trifolium repens, and Medicago lupulina,* were chosen based on their results. As all these plants are fast-growing, easy to grow and propagate, and primarily self-sustaining, they can be used for phytostabilization. The values of accumulation factors in the six plants were below unity, so they were considered excluders and pseudometallophytes. Nevertheless, two plant species, *M. lupulina* and *L. hispidus* displayed translocation capacity and can be potential candidates for future studies.

Phytovolatization

Phytovolatilization is the process by which plants or their associated microbes volatilize contaminants. This is followed by their translocation to stomata and, sometimes, to the bark and stem tissues to be released into the atmosphere (Limmer & Burken, 2016). This can occur in two ways, direct and indirect, as shown in Fig. 3. In the presence of hydroxyl radicals, volatilized substances in the atmosphere might be damaged or oxidized. This is most commonly used for pollutants treated by conventional air sparging, including benzene, toluene, ethylbenzene, and xylene (BTEX) compounds, trichloroethylene (TCE), vinyl chloride, and 20 carbon tetrachloride, which have a Henry constant value KH >10 atm m^3 air, but not for compounds such as phenol, which have low volatility with a Henry constant value KH of 10 atm m^3 air (Kamath et al., 2004). Examples of uses of phytovolatilization include volatilization of trichloroethylene by poplar trees, methyl tertiary butyl ether (MTBE) by weeping willows, and selenium by Indian mustard (Souza et al., 2002; Yu & Gu, 2006; Doucette et al., 2013). Although this process has sparked concerns because of the risk of air pollution, it is not the primary dissipation pathway for most contaminants (Limmer & Burken, 2016).

Evapotranspiration

The process of evapotranspiration is a type of containment that uses the vaporation and vaporization of water to control groundwater hydraulics. Pollutant containment is achieved through plume capture by the formation of a depression zone inside the aquifer by plant roots, which prevents pollutants from migrating off-site and downward (Kamath et al., 2004). In arid and semi-arid locations, evapotranspiration by natural vegetation is effective, but it might also occur in different regions if climate and other conditions are taken into account (USEPA, 2000). Phreatophyte trees, such as poplar, eucalyptus, and river cedar, are examples of plants that take this approach, with deep roots that can transpire 200–1100 liters of water every day (Morikawa & Erkin, 2003).

A study by Valujeva et al. (2018) tested phytoremediation as a technology for environmental protection and for preventing the streaming of pollutants into hydrological systems. Their findings showed that the most effective plants for phytostabilization included Alfalfa, Salix, and Poplar species. They recommended that biomass taken from petroleum oil-contaminated sites should be composted or burned, whereas biomass taken from a mixture of contaminants (such as oil products and heavy metals) should be dried and transferred to a waste incineration facility.

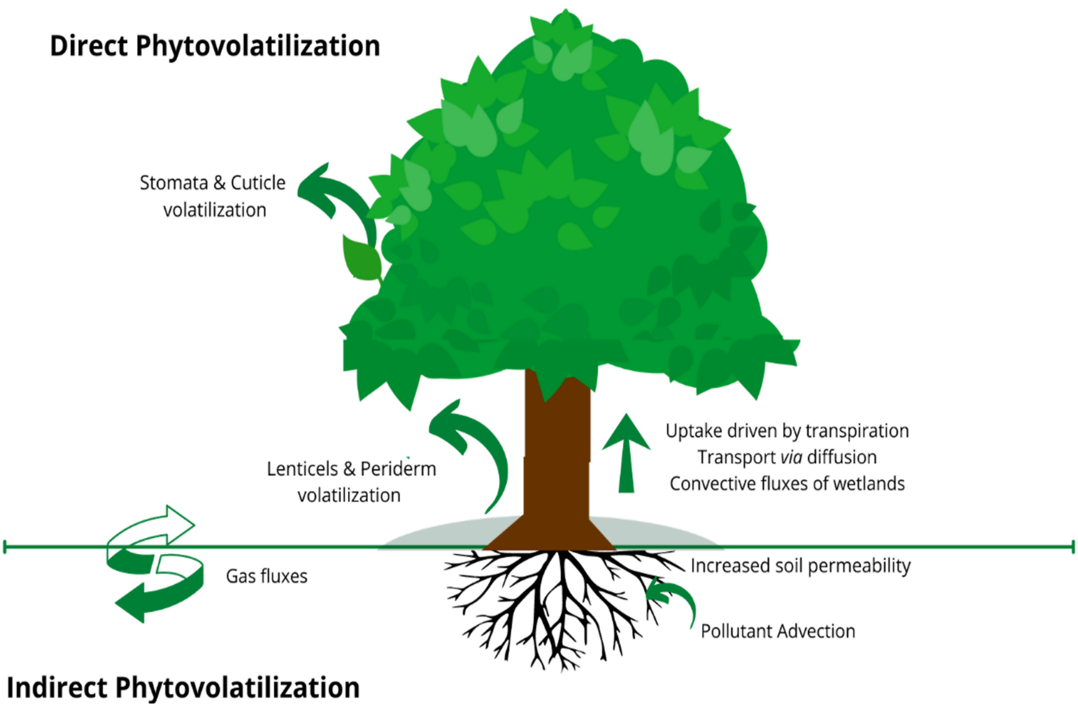


Fig. 3. Direct and indirect phytovolatilization **[Plants are able to interact with various organic compounds, thus affecting the fate and transport of several environmental pollutants. Volatilization of organic compounds could occur via leaves or stems/barks (direct phytovolatilization) or via soil due to plant root activities (indirect phytovolatilization)]**

In the study of Weyens et al. (2010), inoculating yellow lupine with the engineered endophyte *Burkholderia cepacia* resulted in improved phytoremediation potential of volatile organic pollutants and toxic metals from contaminated soils and groundwater by different mechanisms, including evapotranspiration. Because of the decreased enzyme activity involved in antioxidant defence in the roots, the experiment resulted in lower Ni and trichloroethylene (TCE) phytotoxicity. The evapotranspiration of TCE decreased five times faster than Ni uptake after inoculation of the endophyte. In 2003, Matthews et al. (2003) demonstrated that deep-rooted poplar trees take up and degrade groundwater pollutants such as trichloroethylene. The trees evapotranspire water from the shallow polluted aquifer and prevent the downgradient migration of polluted water. The success of phytoremediation in capturing the polluted water was directly proportional to the aquifer's horizontal conductivity, saturated thickness, and groundwater gradient.

An important field of phytoremediation is remediating landfill leachate in municipal landfill sites. This leachate is generated by the decomposition of landfilled organic waste and precipitation percolating through the waste material, which has to be treated before its outflow into the environment. Studies by Dimitriou et al. (2006), Justin & Zupančič (2009), and Aronsson et al. (2010) proved that willow plantations established on the restored cap of landfills can decrease leachate formation due to high evapotranspiration, whereas nutrients from the leachate can be taken up by willows or retained in the soil–plant system.

Phytoaccumulation

Also known as phytoextraction or phytomining, this refers to the removal or uptake of pollutants from the contaminated matrices and their translocation into the harvestable organs of the plants (Suman et al., 2018). It is primarily used on sites with inorganic contaminants, although it may be effective as part of an integrated remediation strategy for sites (soils, sediments, sludges, and to a lesser extent, water) that are co-contaminated with PHC and chemical contaminants, especially heavy metals, even though it is common (Pivetz, 2001; Solomou et al., 2022). As this mechanism requires concentration or accumulation rather than breakdown, this strategy involves the plant roots extracting inorganic pollutants from soil and water and translocating them to the plant shoots, followed by plant harvest for disposal or recycling. Silver, cadmium, cobalt, chromium, copper, mercury, manganese, molybdenum, nickel, lead, and zinc, as well as metalloids such as arsenic and selenium, can all be remediated (Bani et al., 2010; Zhang et al., 2010; Zhang et al., 2011; Yang et al., 2017; Çelik et al., 2018; Siraj et al., 2022).

The process of uptake could be comparable to that of metals or nutrients necessary as co-factors for enzyme activity, for example. After intake, they are retained in vacuoles after intake to protect the plant from their damaging effects. Regarding the Chernobyl nuclear power plant incident in Ukraine, such plants were said to be used to clean up the environment (Morikawa & Erkin, 2003; Wenzel, 2008; Macek et al., 2009). Owing to their poor biomass formation, most hyperaccumulator plants are unsuitable for phytoremediation methods (Grzegórska et al., 2020). The rhizosphere activity, microbial biomass, and metabolism are all influenced by plant biomass production, particularly in the root system (Xiong et al., 2021). The efficacy of accumulation is another key parameter in the selection of a good candidate for phytoremediation. Water plants, microalgae, root filters, and immobilized bacteria are all good alternatives for contaminated water (Yan et al., 2020). Rhizofiltration, similar to phytoaccumulation, is a concentration technique that involves root accumulation and harvesting in hydroponic conditions. This is mostly used to cleanse contaminated water, as documented by the US Department of Energy for radionuclide-affected locations (USEPA, 2000). The phytoremediation of radionuclides has become increasingly important because of their long half-life and their possible entrance into the food web after their accumulation in water and soil. There is a wide range of plant species that can remediate radionuclides with efficient phytoextraction potential (Hegazy & Emam, 2011; Yan et al., 2021). As reported by Hegazy et al. (2013), three black sand plant species—*Rumex pictus*, *Senecio glaucus*, and *Cakile maritima*—were found to be effective candidates for the phytoremediation of radionuclides as they displayed tolerance to the high levels of uranium and thorium accumulation.

The investigation of Qi et al. (2015) on 26 cultivars of wheat, barley, and oats for their ability to accumulate and tolerate Sr. Among the tested cultivars, naked oats and barley showed a significantly higher accumulation of Sr than wheat. Naked oat grains had the highest concentration of accumulated Sr, indicating the plant as a potential candidate for Sr phytoremediation from the polluted soils. Similarly, the soybean plant comparison was tested and the high capabilities of Sr uptake and accumulation were observed, with no toxic effects to the plant noted; the physiological parameters and the plant growth were not affected. Likewise, the *Arabidopsis thaliana* accession Ler-1 showed no sign of Sr toxicity owing to tolerance of up to 1mM Sr (Kanter et al., 2010; Gupta et al., 2018). Several studies mentioned in Yan et al. (2021) investigated the potential of different plant species to remediate radionuclides. Among those studies, *Amaranthus retroflexus* was found to be a potential candidate for the phytoremediation of radionuclide-contaminated soils containing Cs and Sr, whereas *Helianthus annuus* was found to be capable of phytoremediation of soils and water contaminated with Cs, I, Sr, U, and Ra.

The merits of this approach include the use of harvested plants as a resource, such as biomass containing the important mineral, selenium, as animal feed, whereas the drawbacks include the slow growth rate of metal accumulators, small biomass, shallow root systems, phytotoxic effects of pollutants, and the need to harvest and recycle plant biomass. As reported by previous studies (Macek et al., 2009; Megharaj et al., 2011; Zhang et al., 2011; Jadeja & Batty, 2013; Fasani et al., 2018), the phytoaccumulation procedures include the utilization of artificial wetlands and lagoon systems, as well as the use of transgenic plants to meet phytoremediation needs.

Genetically modified plants for phytoremediation

Genetically engineered plants are used as a promising tool for improving phytoremediation abilities. Although phytoremediation is considered ecologically and economically friendly, it has some limitations, including the low removal rates and inadequate tolerance of the plants to the pollutants (Ozyigit et al., 2020). Developing transgenic plants with improved phytoremediation abilities pshouldrovide a solution to overcome the weakness of conventional plants that are used to remediate environmental pollutants (Gunarathne et al., 2019). Genetically engineered plants for phytoremediation purposes were first developed to enhance the tolerance of toxic metals (Aken, 2008). The phytoremediation of toxic metals can be improved by overcoming the limitations of producing low plant biomass and the limited efficiency of particular plant species for phytoremediation (Suman et al., 2018). The genes that are involved in the translocation, detoxification, acquisition, and sequestration of heavy metals have been identified in different organisms, including higher plant species, bacteria, and yeast. These genes can be transferred and overexpressed in plants that have phytoremediation potential. The produced transgenic plants can overexpress proteins that are important in pollutant assimilation and chelation, as well as membrane transport (Pilon-Smits et al., 1999; Maestri & Marmiroli, 2011; Shim et al., 2013).

The remediation process of organic pollutants can be improved by enhancing several mechanisms included in in-planta and ex planta processes in the phytoremediation environment. The uptake of the organic pollutants and their subsequent diffusion to the plant organs, sorption and sequestration, or/and transformation are included as the in planta processes, while the ex planta processes include the degradation that occurs *via* the rhizospheres' microbial activity or the protein and co-factor excretion that results in non-specific activity (James & Strand, 2009). The plants developed for organic pollution phytoremediation were modified to remediate halogenated and explosive pollutants. Currently, a wide variety of applications exist for transgenic plants in organic pollution phytoremediation, such as pesticides, explosives, organic hydrocarbons, phenolics, and organic solvents (Azab et al., 2018). The transgenes present in these plants are responsible for enhancing plant tolerance to pollutants or increasing metabolic activity under pollution stress (Mishra et al., 2020).

The genetic manipulation of enzymes involved in phase I and phase II of xenobiotic metabolism is considered an important approach to enhancing the phytoremediation of organic pollutants. Dioxygenases or cytochrome P450, as well as glutathione-S-transferase, are implicated in the enhancement of organic contaminant phytoremediation (Maestri & Marmiroli, 2011; Mishra et al., 2020). Other approaches target specific types of pollutants, such as the manipulation of laccases and peroxidases, to remediate phenolic compounds and nitroreductase or pentaerythritol tetranitrate reductase for the removal of TNT (James & Strand, 2009; Aken, 2009). The expression of the human P450-1A2 gene enhanced plant tolerance and detoxification to the herbicides chlortoluron, linuron, and simazine in the transgenic *Arabidopsis thaliana* (Kebeish et al., 2014; Azab et al., 2016, 2018). Tolerance towards chlortoluron and isoproturon was reported by using ginseng-derived CYP736A12 and CYP76C1 genes that are overexpressed in *Arabidopsis thaliana* (Hofer et al., 2014; Khanom et al., 2019). Other studies (Buono et al., 2020; Mishra et al., 2020) reported the enhancement of herbicide tolerance and metabolism in different plant species using cytochrome p450 transgenes.

Plants and associated microorganisms in bioremediation

Recent advancements in bioremediation technology and understanding have introduced the use of plants along with microbes for the remediation of petroleum hydrocarbons and other organic contaminants. This can be termed bioaugmentation-assisted phytoremediation (BAP) (Auti et al., 2019). The interactions of microbes with plant systems, both above ground and in the soil, are important for plant productivity and growth in natural ecosystems and agriculture. These interactions are significant for determining the organic pollutants' fate in the plant soil system. Microbial activity and microbial degradation efficiency are measured by the ability to biotransform petroleum hydrocarbons in soil (Jacoby et al., 2017). Improved growth-linked mineralization efficiency is provided by the plant–microbial system in the rhizosphere microbiome or root zone for organically contaminated sites (Azaizeh et al., 2011). The interactions of microbes with plants are affected by the components of the ecosystem and by the development of alterations in the physical environment. The synergetic effect between the microbes in the rhizosphere and plants can notably increase the success of the remediation of petroleum hydrocarbons in the soil. Rhizosphere bacteria are known to be a heterogeneous group of bacteria associated with the roots and on the surface of the roots that improve the quality and extent of plant growth in a direct or/and indirect way. Petroleum contaminants that are present in the soil are degraded by the plant-associated microbes that can include rhizospheric bacteria. The internal tissue of almost all plants is colonized by endophytic bacteria that play an important role in promoting plant yield and growth; this is considered tp be a synergetic relationship (Tiwari et al., 2013). It was reported by Zhang et al. (2012) and Dzionek et al. (2016) that the degradative potential of microbes found in the rhizosphere may be increased by plants in several ways, such as increases in PHC-degrading microbes, the densities of microbial population, the expression of catabolic genes, catabolic genes horizontal transfer, and enhancement of hydrophobic hydrocarbon bioavailability. Plants that secrete organic compounds can induce microbes to degrade PHC by different mechanisms, including efficient microorganism attachment on the plant surface, polluted soil aeration, and the availability of organic pollutants and nutrients transport, even though it was reported that plants rarely have the potential for effective bioremediation of PHC-polluted soils (Gkorezis et al., 2016). Some petroleum hydrocarbon-degrading microbes associated with plant use, also known as plant growth-promoting rhizobacteria (PGPR), have several advantages, including the ability to invert transformation and reduce residual pollutant risks. The plant–microbial system offers higher remediation efficiency than phytoremediation only. During bioremediation that combined bio-stimulation and phytoremediation mechanisms, the organic compounds secreted from plant roots enhanced microbial activity and PHC-degrading microbes; subsequently, the microbial mineralization of organic pollutants in contaminated soils was enhanced (Mohsenzadeh et al., 2010; Zhang et al., 2010; Auti et al., 2019). Plant roots, which are involved in water and nutrient uptake and anchorage, are biochemical units that regulate numerous plant–soil interactions, including mutualistic relationships with beneficial endogenous microbes such as mycorrhizae, rhizobia, endophytes, and PGPR (Tiwari et al., 2013). Plant exudation is the primary influence that affects PHC degradation in the rhizosphere. Various compounds are exuded by plant roots into the rhizosphere, such as organic acids, phenolics, amino acids, and sugars (Puškárová et al., 2013). Plant roots secrete biochemical compounds that are divided into two categories based on their molecular weight: (i) Low-molecular-weight compounds such as phenolics, amino acids, monosaccharides, and aromatic and aliphatic compounds; and (ii) High-molecular-weight compounds such as proteins and polysaccharides (Kumar & Goel, 2019). Plant roots secrete organic acids, such as the intermediates of the citric acid cycle, including malonic, citric, oxalic, fumaric, malic, and succinic acids; these are involved in various processes such as PHC microbial degradation in the soil. The secreted organic acids change the rhizosphere's chemical composition, and as a result, the bioavailability of organic pollutants in soils is changed (Ite et al., 2013). This process is either enhanced directly by changing the soil conditions, including the characteristics of the soil surface and the soil pH, or indirectly by promoting the indigenous PHC-degrading microbial communities. Some soil microbes can mineralize root exudates while being used as growth substrates, which can further act as co-metabolites for the persistent PHC contaminant degradation (Correa-García et al., 2018).

Several studies have demonstrated the potential of certain microbes and plant combinations for the enhancement of the PHC biodegradation process. The inoculation of PGPR in combination with arbuscular mycorrhizal fungi (AMF) in soil contaminated with petroleum hydrocarbons and planted with *Avena sativa* increased plant dry weight and stem height compared to uncontaminated soil. This combination increased the plant's tolerance to crude oil pollution by augmenting the activities of enzymes and decreasing the level of MDA. Further, they contributed to improving the soil quality by increasing the activities of the soil enzymes urease, dehydrogenase, and sucrase (Xun et al., 2015).

The study of Bakaeva et al. (2020) on some selected *Pseudomonas* strains, characterized by their high biodegradation ability of crude oil (about 70%) and intermediate *in vitro* auxin production in the presence of oil, can overcome the inhibitory effect of crude oil stress on barley plants. In the presence of crude oil pollutants, the bacterial strains *P. plecoglossicida* 2.4-D and *P. hunanensis* IB C7 increase the seed germination percentage and have a positive effect on further growth of the plant seedlings when the contamination reaches 8% oil by mass.

Similarly, Das & Kumar (2016) found that seeds of *Withania somnifera* primed with biosurfactant produced PGPR *Pseudomonas* sp. AJ15 produced plants characterized by high values of shoot and root length, carotenoids and chlorophyll pigments, and germination percentage under various levels of crude oil contamination compared to non-primed seeds.

Environmental factors affecting crude oil remediation

Several studies and successful applications have been conducted on the remediation of crude oil pollution in soils and waters. Phytoremediation by higher plants and bioremediation including plants and microorganism techniques were applied. Microbial activity can be affected by several factors (Fig. 4); however, this article focuses on the major factors, namely temperature, salinity, oxygen, pH, water, bioavailability, and nutrients. Microorganisms can acquire catabolic activity by changes in genetic makeup, the induction of certain enzymes, and eclectic enrichment capable of successful biodegradation. The chemical makeup of petroleum hydrocarbons is a key and influential element of biodegradation (Al-Hawash et al., 2018).

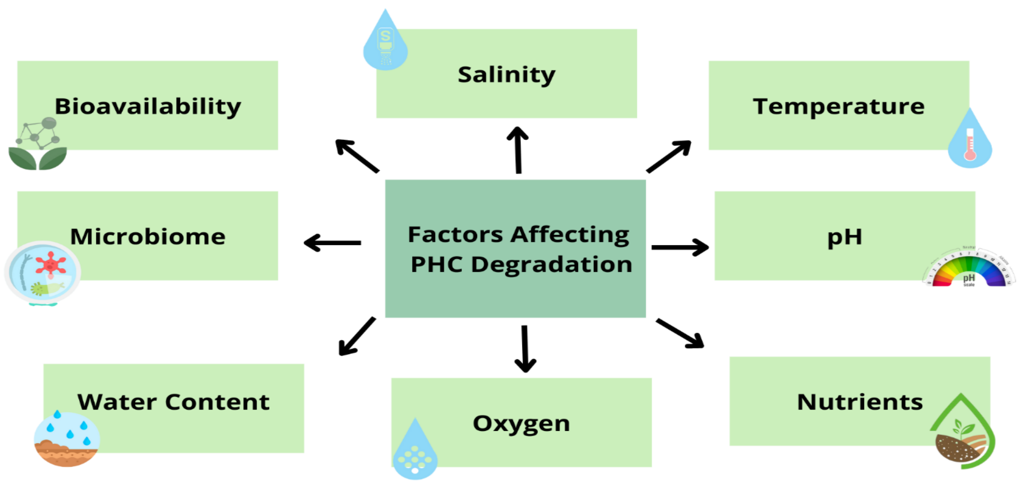


Fig. 4. Environmental factors affecting the biodegradation of petroleum hydrocarbons

Temperature

Temperature is one of the most important elements influencing biodegradation as it can change the physical and chemical properties of PHC (Al-Hawash et al., 2018). According to Bisht et al. (2015), the degradation rate is generally reduced at low temperatures as it is believed to reduce enzymatic activity rates. Al-Hawash et al. (2018) mentioned that the highest rate of PHC was obtained at elevated temperatures, from 30°C to 40°C.

Coulon et al. (2005) conducted a study in the Kerguelen Archipelago using sub-Antarctic soil intentionally polluted with diesel or crude oil to assess the potential of a bioremediation strategy under different temperature conditions. Soils responded favorably to temperature increases between 4°C and 20°C, as well as to the addition of a commercial oleophilic fertilizer containing N and P. Both variables increased the abundance of hydrocarbon-degrading microbes and the breakdown of total petroleum hydrocarbons (TPH). Aung et al. (2018) reported that the bioremediation effectiveness of petroleum hydrocarbons was greatest in summer, followed by spring, autumn, and winter. In summer, alkanes could be significantly degraded after 28 days of incubation, whereas no noticeable degradation was observed in winter.

Salinity

Al-Hawash et al. (2018) illustrated a positive association between salinity and the mineralization rates of PAH in estuarine sediments. According to Qin et al. (2012), salinity has a significant impact on bioremediation and biodegradation processes and microbial growth and diversity. They elaborated their findings, stating that moderate salinity promoted biodegradation in the initial period, and the addition of a microbial consortium was ineffective in increasing the degradation rate of petroleum hydrocarbons. After approximately 4 weeks, treatments with longer leaching periods had a higher degradation rate, with a maximum value of 42.36%. As bioremediation progressed, dehydrogenase activity was increased and a positive association was seen between dehydrogenase activity and the degradation rate of PHCs. As pointed out by Ebadi et al. (2017), salinity inhibits the activity of many important enzymes involved in hydrocarbon remediation.

pH

The pH of the environment has direct and indirect effects on various processes, such as cell membrane transport, catalytic reaction balance, and enzyme activities (Frankenberger & Johanson, 1982; Angelova et al., 2018). Soil organic C, total N, available N, and total P decrease with aridity from dry sub-arid to semi-arid to arid sites. In contrast, soil pH increases with aridity. Hence, knowing the type of soil and how it affects pH will help achieve the optimal pH for enhancement of the bioremediation process (Jiao et al., 2016). Biodegraders such as *Methylobacterium mesophilicum* and *Nocardia otitidiscaviarum* were optimum for both growth and degradation at a pH of 7 (Vyas & Dave, 2007). Koolivand et al. (2022) found that the maximum degradation efficiency of petroleum oil (1%–3% concentration) obtained after 7 days was 72%–75% at pH 7. They also reported that the effect of pH on the growth of oil-degrading bacteria and petroleum oil degradation (1% v/v concentration) was a significant element in determining oil-degrading bacterial metabolism and PHC solubility.

Nutrient availability

Nutrients are important in the bioremediation process as they are a key factor for effective biodegradation of pollutants, as microorganisms require nutrients for their metabolism and growth. Organic matter plays an important role in enhancing the soil properties of promoting the growth and activities of soil microbes and is considered a key source of nutrients (Karthikeyan & Kulakow, 2003). The nutrients include carbon, nitrogen, iron, and phosphorus (Kalantary et al., 2014; Al-Hawash et al., 2018). Owing to rapid microbial metabolic activities, organic carbon matter accumulation in hydrocarbon contaminated areas results in the rapid depletion of inorganic nutrients, such as nitrogen, phosphate, and potassium, limiting the rate and degree of hydrocarbon biodegradation and biotransformation (Kebede et al., 2021). Oil spills lead to a significant increase in carbon and a reduction in nitrogen and phosphorus levels in both marine and freshwater ecosystems (Venosa & Zhu, 2003). In marine environments and wetlands, nitrogen and phosphorus levels are low. In the study of Hesnawi & Adbeib (2013), nutrient addition was necessary to promote the biodegradation of contaminants. Therefore, supplementing contaminated sites with the optimal level of nutrients (N and P) is indispensable for effective hydrocarbon biodegradation. Moreover, the concentration of excess nutrients may inhibit the activity of biodegradation. As reported by Zafra & Cortés-Espinosa (2015), pollution levels exert selective pressure on microorganisms that degrade petroleum. Increased levels of PAH inhibited the growth of microorganisms with acquired resistance to PAH, causing a change in cell membrane structure, sporulation, and mycelia pigmentation. In addition, Balaji et al. (2014) investigated different carbon sources for lipase production in *Mucor racemosus*, *Penicillium chrysogenum,* and *Lasiodiplodia theobromae*, and found that sucrose and cellulose promoted the maximum activity in those species.

Oxygen

Bioremediation may occur in either aerobic or anaerobic conditions (Vidali, 2001). Microbial consortia from soil and sludge are capable of metabolizing unsubstituted and alkyl-substituted aromatics, including benzene, 1,3-dimethyl benzene, acenaphthene, naphthalene, toluene, and xylene. According to Chandra et al. (2013) and Al-Hawash et al. (2018), the aerobic biodegradation of PHC was higher than anaerobic biodegradation. A rate-limiting variable determines the oxygen concentration for PH breakdown in the environment. The availability of oxygen in the soil is determined by microbial oxygen rates of consumption, soil type, and water content, with usable substrates resulting in oxygen depletion (Al-Hawash et al., 2018). Meanwhile, as reported by Chandra et al. (2013), biodegradation can occur in anaerobic conditions. According to Sonawdekar (2012) and Abubakar Clarkson & Isa Abubakar (2015), 3.1mg/mL of oxygen is required for the destruction of 1mg/mL of hydrocarbon pollutants, although this does not take into consideration the entire biomass of potential hydrocarbon-degrading bacteria, and 10%–40% of oxygen is necessary for successful hydrocarbon biodegradation. Hence, aerobic catabolism has a faster rate of biodegradation than anaerobic metabolism. According to reports mentioned by Imron et al. (2019), isolated bacterial strains degraded 20%–25% of the total amount of oil in 10 days under aerobic conditions, but 50 days were required for the same strains to degrade 15%–18% of the total petroleum present under anaerobic conditions, with no or minimal hydrocarbon pollutant degradation in the anoxic soil region. The low oxygen levels in hydrocarbon-contaminated locations disturb the aerobic breakdown pathways, resulting in low removal efficacy.

Substrate water content

The water content of the soil is one of the main parameters that affects the rate of biodegradation of petroleum compounds. The transport medium for soil nutrients and the elimination of bacterial metabolic waste products in soil particles is oil moisture (water film). It has an impact on hydrocarbon bioavailability, aeration status, the nature and number of soluble materials, osmotic pressure, diffusion processes, gas transfer, soil toxicity, and soil pH (Kebede et al., 2021). The porosity and water-holding capacity of the soil are diminished when it contains hydrocarbon pollutants. Soil microbes live in the interstitial water of soil pores; therefore, a lower amount of water implies fewer microbes are present, and a slower rate of biodegradation (Chandra et al., 2013). The availability of water for microbial growth in terrestrial habitats may restrict hydrocarbon biodegradation. Oil sludge biodegradation rates in soil were found to be optimum at 30–90% water saturation. Hence, water availability directly influences the movement and microorganisms' growth (Chandra et al., 2013; Al-Hawash et al., 2018). Extreme moisture levels, alternatively, are detrimental to microbial development and metabolism. This is because, rather than creating anaerobic soil conditions, oxygen diffusion in the soil is reduced and aerobic hydrocarbon breakdown is hindered. To promote microbial activity for hydrocarbon breakdown, sufficient moisture (water availability) in hydrocarbon-contaminated locations is required (Kebede et al., 2021).

Bioavailability and the microbiome

Bioavailability refers to the chemical components in soil that may be taken up or converted by living organisms, as well as the method of influencing the extent and rate of biodegradation by physical, chemical, and microbiological factors. The bioavailable part of the hydrocarbons is the part accessible to microorganisms. Petroleum hydrocarbons have low bioavailability and are classified as hydrophobic organic pollutants. The bioavailability of pollutants is an important aspect controlling their effective biodegradation (Chandra et al., 2013; Al-Hawash et al., 2018).

As bioavailability is a key factor in efficiently biodegrading pollutants, the microbiome at the contamination site is one of the most crucial factors affecting the extent of biodegradation.

In the normal environment, up to 19% of the bacterial community are oil-degrading. Diverse bacterial and fungal species have the potential to degrade or transform PAH. The most well-known hydrocarbon-degrading bacteria belong to the genera of *Pseudomonas, Bacillus, Sphingomonas, Rhodococcus*, and *Alcaligenes* (Ghosal et al., 2016). Microbial communities, metabolic pathways, genes, enzymes, and genetic regulation involved in PAH degradation have been the focus of PAH research to investigate how its abundance and richness are intimately connected to the nature and types of PHC pollutants, as well as the surrounding environmental conditions, including oxygen concentration, pH, salinity, temperature, and nutrient availability (Kebede et al., 2021). In many studies, these strategies have contributed to the identification of the key microbial players, genes, and mechanisms for PAH degradation.

Economics of phytoremediation

Phytoremediation is known to be a cost-effective technology for the remediation of environmental pollution (Gerhardt *et al.*, 2009). Several studies have assessed the costs and economic effectiveness of phytoremediation under different factors that may influence the final cost of this technology (Compernolle et al., 2012). Such factors include the pollutant type, size, and concentration of the polluted site, climate, vegetation cover, and agronomical practices. Different mixtures of highly hydrophobic to highly mobile compounds are found in different polluted sites (Kamath et al., 2004). Design, installation, annual operation, monitoring, and maintenance are usually the main items included in the overall cost of bioremediation (Wan et al., 2016). A complete system design is necessary for the application of phytoremediation techniques, which includes treatability studies, pilot experiments to examine the polluted area, toxicity levels of tpollutants, and plant species appropriateness to ensure effective treatment. Plant selection and planting procedures are an additional cost in the installation phase (U.S. EPA, 2000, Zhang et al., 2021). Plant species account for approximately 1%–2% of the total cost of bioremediation installation. Planting and maintenance procedures may require extensive physical labour or heavy equipment (ITRC, 2009).

Phytoremediation has up to approximately 90% lower costs compared to other conventional technologies. The conventional treatment cost of nuclear hazardous waste is estimated at US $400,000 per acre for a 50 cm remediation depth, whereas phytoremediation costs US $60,000–100,000 (Cristina Negri & Hinchman, 1996). Other measures, such as the selection of tree species and vegetation types, can help to reduce costs. Trees are the cheapest plant species for bioremediation because they have a deeper root system, are ideal for sites with low fertility and poor structure, and have rapid growth and high transpiration rates, allowing them to remediate higher quantities of contaminants (Thawale et al., 2006). In a two-year study by Wan et al. (2016), the phytoremediation of cadmium-, arsenic-, and lead-contaminated soil was investigated to determine the required parameters in the remediation process. The study revealed a total cost of US $\$37.7/m^3$ or US $\$75,375.2/hm^2$ with 53.98% and 46.02% for the operational and initial capital costs, respectively. The highest costs were for fertilizers and the infrastructure, including bridges, culverts, and roads. This was mainly attributed to the serious contamination and slow economic development. The study also reported a lower cost for phytoremediation in comparison to other remediation techniques. A cost-effectiveness analysis was conducted by Chen & Li (2018) on the three methods of remediation, phytoextraction, soil washing, soil excavation, and disposal. Different scenarios were considered, including different site scales, contamination levels, soil metals, and soil texture. The study concluded that phytoextraction was more cost-effective for slightly contaminated soils. Zhang et al. (2021) reported that the cost of heavy metal phytoremediation of *Sedum alfredii* monoculture was US $\$15.4m^{-3}$ with a soil depth of 20cm. They compared phytoremediation costs to the cost of traditional technologies, such as soil excavation (US $\$500m^{-3}$), soil vapor extraction (US $\$72\ m^{-3}$), soil washing (US $70–200m^{-3}$), and bioremediation (US $\$51m^{-3}$) (Day et al., 1997; Bhuyan & Latin, 2012; Chen & Li, 2018). Compared to traditional methods, the phytoremediation of Pb, Cd, Zn, and Cs has been reported to be cost-effective (Salt et al., 1995; Berti & Cunningham, 1997).

Air sparging, bioremediation, land farming, and phytoremediation are technologies for the remediation of petroleum hydrocarbons. Air sparging is a low-cost technology; it has a low capital cost and a short treatment time. Bioremediation has moderate costs based on the capital outlay and management. Land farming technology also has moderate costs as it has an initial capital outlay. Phytoremediation technology, in addition to its advantage as an ecofriendly and ecologically benign technique, requires a low capital cost to remediate petroleum hydrocarbons (Kujat, 1999; Gerhardt et al., 2009). A cost of US $2500–15,000 per hectare was reported by the USEPA (2000) for PHC phytoremediation, compared to US $7500–20,000 per hectare for *in situ* microbial remediation (USEPA, 2000). Cunningham et al. (1996) reported that the phytoremediation of petroleum contaminants has a lower cost than the traditional methods of phytoremediation, with US $2500–15000ha^{-1} for traditional methods, and US $20,000–60,000ha^{-1} for traditional remediation for 15 cm of soil depth.

Finally, although phytoremediation is considered an eco-friendly and cost-effective technology, it requires a longer duration than other remediation technologies. Generally, bioremediation may extract, degrade, stabilize, and volatilize the contaminants found in soil and water environments effectively, as well as improve the soil conditions and prevent erosion (Compernolle et al., 2012; Sabir et al., 2015; Wan et al., 2016; Chen & Li, 2018).

Bioremediation and restoration of ecosystems

Bioremediation and associated phytotechnologies have been used successfully throughout the world to bridge the gap between ecological degradation and ecosystem restoration along urban-to-rural gradients (Zalesny et al., 2019). Many phytotechnologies attempt to enhance water quality by targeting escaping contaminants in groundwater or to treat contaminated water before it passes into the water supply (ITRC, 2009). Some phytotechnologies, such as wetland systems, have the unique ability to partially or completely eliminate pollutants that would otherwise require a lot of energy or are not receptive to other technologies (Schröder et al., 2007).

Air pollution is a massively complex issue, despite the advances of phytotechnology to eliminate airborne pollutants. Plants are generally acknowledged as a means to improve air quality, especially in urbanized areas (Baumgardner et al., 2012; Ismail et al., 2017). The current research has focused on achieving a detailed explanation for how plants inhibit pollution transit or trap airborne pollutants (Lee et al., 2020). Phytotechnology methods could be employed to evaluate and map exposure pathways of contaminants (Henry et al., 2013).

Potential vapor intrusion (VI) exposure pathways that connect groundwater to indoor air exposure were identified using phytoremediation technologies. As plant roots occupy similar geologic spaces to foundations, there is a link between VI and plant uptake (Henry et al., 2013). Phytotechnology is a technology-driven science that may be used to protect not only the environment, but also individuals from the detrimental effects of dangerous pollutants..

Conclusions and Future Perspectives

Petroleum hydrocarbons released from crude petroleum oil and associated toxic metal pollution have environmental impacts and affect living organisms at different levels of organization, from the cellular level to the ecosystem level. At the molecular level, the banding patterns of markers revealed elevated polymorphism with a trend toward pollution level-dependent alteration in DNA profiles and decreased genomic template stability as the petroleum pollution level increased. However, there is a need for future research aimed at understanding the extent of the damage and effective methodologies for the recovery of polluted ecosystems using phytoremediation technologies. The thermal, mechanical, and chemical methods applied for remediation to recover contaminated sites are expensive, energy-consuming, and environmentally disruptive.

The promising behavior of some plant species that were used in the phytoremediation of petroleum hydrocarbon-polluted areas has been demonstrated. The development of plants raised in petroleum-polluted sites was affected at different growth stages. Seed germination, as the primary developmental step in plant establishment, growth inhibition, and disturbed metabolic pathways are among the processes most affected by petroleum oil pollution. The water-repellent characteristics of hydrocarbons acts as a physical barrier, slowing the development of the plants due to a delay in or prevention of water and oxygen reaching the seeds and developing plants. In addition, small hydrocarbon molecules can enter and pass through cell membranes, leading to reduced membrane integrity or even cell death.

Bioremediation enhances crude oil degradation from contaminated soils. The plant–microbial systems provide more efficient growth-linked mineralization of organic contaminated sites in the root zone or rhizosphere microbiome. Plant–microbe interactions are obstructed by changes in the physical environment and the ecosystem components produced by pollution. Crude oil degradation and removal from the polluted soils or growth media results from the activities of naturally occurring soil microorganisms. The diversity, activity, and growth of microorganisms in the plant rhizosphere environment are enhanced, most likely due to plant root exudate promotion of the soil microorganisms' growth, as they contain nutrients and energy sources.

Several environmental factors may affect the biodegradation or removal of petroleum hydrocarbons and should therefore be investigated. These factors include temperature, type, and concentration of the pollutants, salinity, pH, nutrient, and oxygen availability, as well as the diversity of microorganisms in the polluted sites. Therefore, some environmental conditions must be adjusted to improve the process of bioremediation, such as improving aeration and optimizing the pH. Further research is needed to screen and investigate the tolerance of plant species to crude oil pollution at various organizational levels, which should take into account food chains and food webs for the better selection of plants suitable for remediation and restoration of crude oil-polluted ecosystems.

List of abbreviations

ABA	Abscisic acid
AMF	Arbuscular mycorrhizal fungi
BAP	Bioaugmentation-assisted phytoremediation
CA	Chromosomal aberrations
ECEC	Effective cation exchange capacity
GTS	Genomic template stability
MDA	Malondialdehyde
MI	Mitotic index
MN	Micronuclei frequency
MTBE	Methyl tertiary butyl ether
PAH	Polycyclic aromatic hydrocarbons
PGPR	Plant growth-promoting rhizobacteria
PHC	Petroleum hydrocarbons
SCGE	Single-cell gel electrophoresis
TCE	trichloroethylene
TPH	Total petroleum hydrocarbons
VI	Vapor intrusion

Competing interests: The authors report no conflicts of interest regarding this work.

Authors' contributions: Ahmad K. Hegazy conceived the idea of the paper . Data was collected by all the authors. Zahra S. Hussien, Nashwa Hamido and Ahmad K. Hegazy wrote the manuscript. The manuscript was edited and revised by Ahmed K. Hegazy, Nermen H. Mohamed, Gehan Safwat and Mohamed A. El- Dessouky.

Ethics approval: Not applicable.

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المعالجة النباتية للتلوث بزيت النفط الخام: دراسة مرجعية

زهرة صالح حسين[1]، نشوى حميدو[1]، أحمد كامل حجازي[2]، محمد علي الدسوقي[3]، نرمين حفني محمد[4]، جيهان صفوت [1]

[1]كلية التقنية الحيوية- جامعة اكتوبر للعلوم الحديثة والأداب- مدينة السادس من أكتوبر- الجيزة - مصر، [2]قسم النبات والميكروبيولوجي- كلية العلوم - جامعة القاهرة- الجيزة- مصر، [3]قسم الكيمياء- كلية العلوم- جامعة القاهرة- الجيزة- مصر، [4]معهد بحوث البترول المصري- مدينة نصر- القاهرة- مصر.

ازداد التلوث البيئي الناتج عن الهيدروكربونات البترولية نتيجة للاستخدام المتزايد للبترول والزيادة في عمليات الاستكشاف والإنتاج والنقل والتصنيع الخاصة بالزيت الخام، وترتب على ذلك ضرورة إيجاد طرق فورية لمعالجة هذا التلوث. قد تكون التقنيات التقليدية المستخدمة لتنظيف هذه الملوثات في كل من النظم البيئية المائية والبرية فعالة ولكنها مكلفة وتتطلب طاقة بشرية ومعدات متخصصة. تعتبر تقنيات المعالجة بالمركبات الكيميائية مثل المشتتات والمنظفات والمستحلبات والمواد الحيوية المؤكسدة للتربة كأكثر التقنيات استخداما ولكن يترتب عليها عواقب بيئية سلبية. تهدف هذه الدراسة إلى عرض الاليات النباتية / البيولوجية المختلفة لمعالجة آثار التلوث بالنفط الخام والتي تعتبر من التقنيات الناشئة الفعالة من حيث التكلفة وتأثيرها على البيئة. يتضمن البحث مناقشة تأثير التلوث البترولي على النظم البيئية وعواقبه، خاصة التي تشمل الطفرات اللاجينية وطفرات الحمض النووي بالإضافة الي العوامل المؤثرة على المعالجة الحيوية للهيدروكربونات البترولية. كذلك تمت مقارنة اقتصاديات المعالجة بالطرق النباتية والطرق التقليدية لاستعادة المواقع الملوثة، علما بأن اليات المعالجة النباتية للملوثات تتم عن طريق تكسيرها، وتثبيتها، وتحويلها كيميائيا داخل النبات، والتخلص منها عن طريق التبخر،