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Title

Plant-microorganism interactions in the bioremediation of soils polluted by petroleum hydrocarbons

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Most of all, we thank the Almighty God, who gave us strength and health while we are doing this work

We humbly dedicate this modest work to our parents

We also dedicate this work to our family and friends who gave us their mental and intellectual reinforcement during our process.

الملخص

تعد الهيدروكربونات البترولية من أهم مصادر التلوث البيئي على المستوى العالمي. حيث ان هذه الاخيرة تعيق نمو النباتات وتؤثر على صحة الكائنات الحية الدقيقة الموجودة في التربة .ذلك مما أدى إلى البحث عن طرق للقضاء على هذه الملوثات؛ حيث تعد المعالجة الحيوية من خلال استخدام النباتات والكائنات الحية الدقيقة وسيلة من الوسائل التي تخفف من المشكلة البيئية.

تهدف هذه الدراسة إلى اختبار التفاعل بين النباتات والكائنات الحية الدقيقة لمعالجة التربة الملوثة بالديزل والبنزين، وكذلك اختبار أدائها وكفاءتها.خيث تم اختبار بدور عباد الشمس (*Helianthus annus*) و خمس سلالات بكتيرية (*Pseudomonas aeruginosa*، *Enterococcus Gallinarum*، *Aneurinibacillus migulanus*، *Streptomyces cinereoruber*، و *Lysinibacillus Cavernae*) التي تم عزلها سابقًا من التربة الملوثة بالنفط، بالإضافة إلى استعمال المجموعة، لقدرتها على تحليل زيت الديزل و البنزين (10%) في التربة الملوثة (فردياً ومع النبات)، من خلال مراقبة معدلات تحلل الهيدروكربونات البترولية الكلية (TPH) خلال 21 يوماً.

اظهرت النتائج التي تم الحصول عليها بعد فترة حضانة مدتها 21 يوما أن جميع السلالات المختبرة والاتحاد لديها القدرة على تحليل كل من البنزين وزيت الديزل في التربة بمعدلات مختلفة. ومع ذلك، فإن *Lysinibacillus Cavernae* و*Enterococcus galinarum*، يظهران معدل تحلل أعلى للديزل والبنزين، على التوالي. زيادة على ذلك، فإن معدلات تحلل كلا الملوثين بواسطة جميع الأنواع البكتيرية والاتحاد المقترن بالنباتات أعلى من معدلات تحللها بدون النباتات بشكل عام.

كان لدى *Pseudomonas aeruginosa* و *Lysinibacillus cavernae* أعلى نسبة تحلل للديزل والبنزين على التوالي.

بالإضافة إلى ذلك، أظهرت هذه التجربة أنه تم ملاحظة نمو النبات في الاوساط الزراعية التي تحتوي على تربة معقمة و معالجة بالسلالات البكتيرية *Streptomyces cinereoruber*، *Enterococcusgalinarum*، و المجموعة ، وكذلك في الأوساط الزراعية التي تحتوي على تربة ملوثة و المعالجة بنفس السلالات البكتيرية والاتحاد.

من خلال هذه الدراسة، أثبتنا أن الجمع بين النباتات والبكتيريا يؤدي إلى معدل تحلل أعلى للهيدروكربونات البترولية، وبالتالي توفير علاج أفضل.

الكلمات المفتاحية: الهيدروكربونات البترولية، التلوث، زيت الديزل، البنزين، المعالجة الحيوية النباتية، النباتات، الكائنات الحية الدقيقة، الاتحاد البكتيري.

Abstract

Petroleum hydrocarbons are the most significant sources of environmental pollution on a global scale. Petroleum products hamper the growth of plants and affect soil microflora. This has led to a search for methods to eliminate such pollutants; Phytoremediation describes the treatment of environmental pollutants through the use of plants and microorganisms that mitigate the environmental problem.

This study aim to test the interaction between plants and microorganisms for the treatment of polluted soil with diesel and gasoline, as well as their performance and efficiency.

Sunflower seeds (*Helianthus annus*) and five bacterial strains (*Pseudomonas aeruginosa*, *Enterococcus gallinarum, Aneurinibacillus migulanus, Streptomyces cinereoruber,* and *Lysinibacillus cavernae*) previously isolated from petroleum contaminated soils, as well as a consortium, were tested for their ability to degrade diesel oil and gasoline (10%) in contaminated soil (individually and with plant), through the monitoring of total petroleum hydrocarbon (TPH) degradation rates during 21 days.

The obtained results after 21 days incubation period, demonstrated that, all the tested strains and consortium have the ability to degrade both gasoline and diesel oil in soil at different rates. However *Lysinibacillus cavernae* and *Enterococcus gallinarum*, demonstrate the higher rate of degradation of diesel and gasoline, respectively.

Furthermore, the rates of degradation of both contaminants by all bacterial species and consortium coupled with plants are higher than their rates of degradation without plants in general. *Pseudomonas aeruginosa* and *Lysinibacillus cavernae* had the highest percentage of degradation of diesel and gasoline, respectively.

In addition, this experiment shows that plant growth was observed in the pots containing sterile soil treated with bacterial strains *Streptomyces cinereoruber*, *Enterococcus gallinarum*, and consortium, as well as in the pots containing polluted soil treated with the same bacterial strains and consortium.

Through this study, we have proven that combining plants with bacteria yields a higher degradation rate of petroleum hydrocarbons, thereby providing better remediation.

Keywords: Petroleum hydrocarbons, pollution, diesel oil, gasoline, bioremediation phytoremediation, plants, microorganisms, consortium**.**

Résumé

Les hydrocarbures pétroliers sont les sources les plus importantes de pollution environnementale à l'échelle mondiale. Les produits pétroliers entravent la croissance des plantes et affectent la population saine de microflore indigène dans les sols. Cela conduit à la recherche des méthodes pour éliminer ces polluants; la phytoremédiation décrit le traitement des problèmes environnementaux par l'utilisation de plantes et des micro-organismes qui atténuent le problème environnemental.

Cette étude vise à tester l'interaction entre les plantes et les micro-organismes pour le traitement des sols pollués par le diesel et l'essence, ainsi que leur performance et leur efficacité. Des graines de tournesol (*Helianthus annus*) et cinq souches bactériennes (*Pseudomonas aeruginosa, Enterococcus gallinarum, Aneurinibacillus migulanus, Streptomyces cinereoruber,* et *Lysinibacillus cavernae*) préalablement isolées de sols contaminés par des hydrocarbures pétroliers, ainsi qu'un consortium, ont été testées pour leur capacité à dégrader le diesel et l'essence (10%) dans des sols contaminés (individuellement et avec plante), à travers le suivi des taux de dégradation des hydrocarbures pétroliers totaux (HPT) pendant 21 jours.

Les résultats obtenus après une période d'incubation de 21 jours ont démontré que toutes les souches testées et le consortium ont la capacité de dégrader à la fois l'essence et le diesel dans le sol à des taux différents. Cependant, *Lysinibacillus cavernae* et *Enterococcus gallinarum* ont démontré le taux de dégradation le plus élevé pour le diesel et l'essence, respectivement.

En outre, les taux de dégradation des deux contaminants par toutes les espèces bactériennes et le consortium couplés avec la plante sont généralement plus élevés que leurs taux de dégradation sans plante. *Pseudomonas aeruginosa* et *Lysinibacillus cavernae* ont montré les taux de dégradation les plus élevé dans le diesel et l'essence, respectivement. De plus, cette expérience montre que la croissance des plantes a été observée dans les pots contenant du sol stérile traité avec des souches bactériennes *Streptomyces cinereoruber*, *Enterococcus gallinarum,* et le consortium, ainsi que dans les pots contenant du sol pollué traité avec les mêmes souches bactériennes et le consortium.

À travers cette étude, nous avons pu démontrer que la combinaison de plantes avec des bactéries produit un taux de dégradation plus élevé des hydrocarbures pétroliers, offrant ainsi une meilleure remédiation.

Mots-clés : hydrocarbures pétroliers, pollution, huile diesel, essence, bioremédiation, phytoremédiation, plantes, micro-organismes, consortium.

Table of content

Table of content

Literature review

Methodology

Results

List of figures

List of tables

List of abbreviations

OD	Optical density.
PAHs	Polycyclic aromatic hydrocarbons.
PH	Petroleum hydrocarbon
TPHs	Total petroleum hydrocarbons.
PGPR	Plant growth-promoting rhizobacteria.
Aw	Water activity.
TPHi	Initial TPH concentrations
TPHr	Residual TPH concentrations

Introduction

Introduction

Petroleum hydrocarbons play a vital role as an energy source and as raw materials for many different industries. Global primary energy consumption in 2009 was 11164.3 million tons of oil (Goeury., 2012). The increasing need for petroleum products in daily life could result in their scarcity and increased expense (Varjani., 2017).

Algeria is ranked third in Africa and 12^{th} worldwide among nations that produce oil. The Sahara region of southern Algeria is known for its robust oil sector, particularly in oil extraction, marshes, development of manufacturing facilities, and refining. In 2018, Algeria produced over 48.3 million tons of oil and in 2019, saw 80 wells drilled as part of exploration drilling activities (Chabi et al., 2023).

However, there is a serious risk associated with petroleum product releases into the environment. They can seriously harm the ecosystem and have an impact on human health as well as terrestrial and marine ecological systems (Kout et al., 2022). In addition, oil can leak into the soil during extraction and transportation, changing the soil's composition and structure, and it can also pose risks to plants animal and human health (Sui et al., 2021).

Biological, chemical, physical, and thermal techniques are performed, both in situ and ex situ, to treat polluted soils. Physico-chemical and thermal methods are more intensive for the soil matrix, requiring greater amounts of energy and higher costs than the biological ones, but allowing for better remediation efficiencies (Caetono et al., 2019). However, biological treatment, including bioremediation using microbial/enzymatic activity, and phytoremediation using plants to treat contaminated soil or water, has proven to be the most cost-efficient and ecologically friendly option available globally (Waychal et al., 2022). In addition, bioremediation technology is widely used because it doesn't change the structure of the soil or produce secondary contamination (Wei et al., 2021).

Although various aspects of phytoremediation involve the plant and its microbiota, rhizoremediation stands out as an integrated plant-microbe endeavor. Rhizoremediation is the degradation of pollutants in the rhizosphere through microbial activity. Plants, through phytodegradation and rhizoremediation, directly break down organic pollutants through their enzymes, promoting the growth of microbial communities in the rhizosphere, which is the primary method for eliminating hydrocarbons (Truskewycz et al., 2019).

In this perspective, this study aims to evaluate the ability of microorganisms, previously isolated from petroleum contaminated soils, to degrade gasoline and diesel in soil. These microorganisms will be tested alone, in consortium and in combination with a plant.

Literature review

Literature review

1. Petroleum hydrocarbons pollution

The name petroleum, often known as crude oil, comes from the Latin Petra and oleum, which mean rock oil. It is a highly complex mixture of hydrocarbon compounds that naturally exists in sedimentary rocks as gases, liquids, semisolids, or solids. It also contains traces of metals and chemicals that contain nitrogen, oxygen, and sulfur (Wante et al., 2021).

Petroleum is a complex mixture made of thousands of compounds, which can be divided into four major fractions: the alkanes, the aromatics, the resins, and the asphaltenes (Godheja et al., 2016).

Petroleum hydrocarbons are formed from the remains of dead plants and animals in the Earth's crust, and serve as a crucial energy source for transportation, industry, and civil society (Pandolfo et al., 2023).

Petroleum hydrocarbon pollution is a serious environmental issue exacerbated by extensive anthropogenic and industrial activities. The increasing demand for petroleum and oil over the past decades has led to significant oil waste releases, contributing to severe environmental pollution (Dhote et al., 2018).

Annually, the projected amount of natural crude oil seepage is 600,000 metric tons, with a 200,000 metric ton uncertainty range. Oil spills are a global problem that affects both industrialized and developing nations. In addition, petroleum products are a major source of soil pollution since they have an impact on all living organisms (Sahu et al., 2020).

Petroleum hydrocarbons are pervasive pollution that enters the environment through a variety of pathways, including unintentional or deliberate spills from oil production platforms, floating and shoreline oil storage facilities, and other industrial operations, as well as combined storm water overflows and passing barges and vessels (Silva-Castro et al., 2013). Unknown-source oil spills also frequently happen in open water, rivers, and navigable coastal waterways. All these activities involve significant risks to the environment, which need to be reduced.

Additionally, the majority of this pollution is caused by waste oil transportation, refining, and exploration, as well as by inadequate waste oil recycling and inappropriate landfill disposal of hazardous oil wastes that are not adequately managed. The number of contaminated places has risen significantly as a result of human activity (Zuzolo et al., 2021).

2. Impact of petroleum hydrocarbons contamination

2.1. Soil

Soil is a fundamental and irreplaceable natural resource, providing a vital link between the components of the environment (air, bedrock, water, and biota). These elements work together to provide food, fuel, and fiber to support living organisms (Adipah 2018)

Soil pollution by petroleum hydrocarbons, including aliphatic, aromatic, heterocyclic, and asphaltene hydrocarbons, is a serious environmental and health issue globally due to their high toxicity and persistence in the environment (Zuzolo et al., 2021). Furthermore, petroleum hydrocarbons are obstructing the pore spaces in the soil, which raises the temperature of the soil and reduces the number of beneficial microorganisms. Moreover, these pollutants prevent plants from absorbing nutrients, which eventually leads to plant death (Nemati et al., 2024). Also, the ecological effects of oil on soil functionality are most notably observed through changes in the activity of soil microorganisms and enzymes (Polyak et al., 2015). Additionally, soil and underground environmental contamination due to petroleum product leaks is a significant issue in the industrial sector (Salanitro., 2001).

Petroleum hydrocarbon contamination of soils and sediments poses a global environmental risk, as aromatic components are refractory in the absence of oxygen, and some fractions are toxic and have a tendency to bioaccumulate in food chains (Ite et al., 2019). It can also migrate from the soil to the groundwater when it seeps into the soil through spills or leaks (Adipah 2018). The health of individuals is significantly impacted by the presence of total petroleum hydrocarbons (TPHs) in the environment (Kuppysamy et al., 2019).

2.2. Plants

Soil pollution has a significant impact on food security because it reduces agricultural productivity and makes crops unsafe for consumption. This dual threat arises from the impairment of plant metabolism and the harm to microbial biomass in the soil (Zuzolo et al., 2021). In addition, plants are directly affected by diesel contamination in the soil, which also prevents appropriate soil aeration and lowers germination rates (Ahmed et al., 2021)

Plants exposed to hydrocarbons suffer from direct toxicity, reduced nutrient and water absorption, and restricted light availability due to oil obstructing their movement through the soil matrix. These factors reduce plant productivity (Truskewycz et al., 2019). Hydrocarbons also prevent plants from growing by blocking or limiting their intake of water and mineral salts, which causes metabolic processes to malfunction. This lack of nutrients and chlorophyll causes stunted growth, distorted roots, leaves, and flowers, which show up as Chloris and necrosis, as well as decreased susceptibility to pests and diseases (Ossai et al., 2020).

Petroleum hydrocarbon contamination of soils and sediment is a global environmental hazard and poses a human health concern because of the refractory character of the aromatic components in the absence of oxygen (Ite et al., 2019). Furthermore, these pollutants are usually toxic and lethal depending on the chemical nature, composition, and properties of the compound fractions, mode of exposure, level of exposure, and time of exposure. The contaminants can cause a range of toxicological health problems for humans and animals (Ossai et al., 2019).

3. Remediation of soil polluted by petroleum hydrocarbons

Soil remediation methods can be divided into three parts (thermal, chemical, and biological), which can be carried out ex-situ or in-situ depending on the type of process (Soleimani and Jaberi, 2014).

3.1. Physicochemical methods

The physicochemical methods encompassed remediation, recovery, and containment technologies that use physical and mechanical barriers to isolate, recover, or separate contaminants in the soil, sediments, surface water, and groundwater.

The physicochemical treatment is mostly done in situ or ex situ and comprises different techniques such as soil isolation, containment booms and skimmers for surface water, physical barriers, surface capping, hydraulic containment, pump and treat, soil vapor extraction, steam stripping, air sparging, soil flushing, vacuum pumping, and steam-induced volatilization (Ossai et al., 2019). Ex-situ techniques rely on low-temperature thermal desorption (LTTD) as a vital process for treating harmful petroleum hydrocarbons this approach involves the application of heat to physically extract and disperse pollutants from excavated contaminated soils (Ambaye et al., 2022). However, chemical processes consist of destroying pollutants or transforming them into compounds that are less harmful to the environment; mainly through chemical reactions occurring between the pollutant and the added reagent (Bouderhem 2017). The chemical treatment methods involve in situ chemical remediation of contaminated environments, such as stabilization, solidification, immobilization, dispersion, emulsification, oxidation-reduction, dehalogenation, activated carbon, and supercritical fluid oxidation (Ossai et al., 2019).

3.2. Biological methods

Bioremediation is defined as the use of biologically mediated processes to detoxify, degrade, or transform pollutants into an innocuous state (Gkorezis et al., 2016). It is a highly attractive, cost-effective, and environmentally friendly technology based on using microorganisms to remove petroleum hydrocarbon contaminants from soil or water (Bidja Abena et al., 2019).

It’s also one of the methods that offer a green technology solution to the problem of petroleum hydrocarbon contamination (Kuppusamy et al., 2020).

Over the past decades, biological methods, such as bioremediation, mycoremediation, and phytoremediation, have been highlighted in several studies (Soleimani and Jaberi., 2014). Furthermore, numerous studies have demonstrated the important role that a variety of microorganisms or microbial communities, including bacteria, fungus, yeasts, protozoa, and algae, play in the biodegradation of contaminants derived from petroleum hydrocarbons (Bekele et al., 2022)

Biological treatment is favored over physicochemical processes because of its feasibility, reliability, and ability to achieve high removal efficiency at a low cost. Its advantages include the simplicity of its low-energy design, construction, operation, and use, and its reliance on biodegradation rather than the accumulation of hydrocarbons or the use of chemical agents. This makes biological treatment a cost-effective method (Al-Hawash et al., 2018). In addition, biological modes of treatment, such as bioaugmentation, biostimulation, and phytodegradation, are emerging as green solutions due to their long-term sustainability and eco-friendliness.

Oil and polycyclic aromatic hydrocarbon (PAH) contaminants can be removed from the environment more reliably by utilizing the synergistic actions of bacteria and plants, known as rhizospheric effects (Dhote et al., 2018).

Different biological approaches exist for remediating TPH contamination, such as biostimulation, bioaugmentation, and phytoremediation, all aiming for the complete mineralization of petroleum hydrocarbons (Zuzolo et al., 2021).

Bioaugmentation process involves introducing hydrocarbon-degrading microbial strains or consortia into polluted environments, including exogenous cultures, autochthonous communities, or genetically engineered microbes with specific catabolic activity (Juhasz et al., 2005). However, biostimulation entails supplying macro- and micronutrients, maintaining temperature, aeration, and pH levels, and amending surfactants to enhance soil conditions and accelerate biodegradation by increasing the growth rate of native microorganisms that break down hydrocarbons. These enhancements significantly improve the bioremediation process's overall efficacy (Alegre et al., 2022).

3.3. Factors affecting bioremediation

The effectiveness of soil bioremediation greatly relies on factors such as the density of the inoculum, its survival rate, colonization ability, competitiveness, microbial activity, and physical diffusion capacity (Bala et al., 2022).

Temperature has a major impact on the bioremediation process because it affects the activity of the bacteria that consume the hydrocarbons and determines their physical state in contaminated areas. Furthermore, temperature affects the solubility of gases, the composition of soil, the rate at which microorganisms develop, the metabolism of those organisms, and the physical and chemical properties of pollutants (Sunita et al., 2017).Additionally, the pollutant's concentration might affect its biodegradation; excessive pollutant concentrations can lead to low microbial activity and insufficient removal efficiency (Silva-Castro et al., 2013).

The oxygen concentration has been identified as the limiting factor for the degradation of PHs in the environment. Soil oxygen levels are influenced by microbial oxygen consumption rates, soil type, water saturation, and the existence of appropriate substrates that might cause oxygen depletion, all of which have an impact on soil oxygen levels. Research has shown that aerobic biodegradation of PHs is more efficient than anaerobic biodegradation (Al-Hawash et al., 2018).

The water activity or water potential (aw) of soils can vary between 0.0 and 0.99, unlike in the marine environment, where water activity remains stable at around 0.98. Moisture is essential for all biological processes as it facilitates the transportation of nutrients, food, and waste materials in and out of microbial cells. In terrestrial ecosystems, the availability of water for microbial growth and metabolism may limit hydrocarbon biodegradation. The ideal moisture ratio depends on the climate and soil type, with ratios ranging from 30% to 90% of the soil (Unimke et al., 2018).

The development and functioning of bacteria are greatly influenced by the availability of nutrients. Therefore, the ability of the microbial population to survive in hydrocarbon-contaminated soil is strongly dependent upon these nutrients' availability.

In a natural environment, hydrocarbon-degrading bacteria are regulated by limits imposed by inorganic nutrients. This suggests that the addition or existence of macronutrients in the contaminated soil, such as potassium (K), phosphorus (P), and nitrogen (N), increases biostimulation. Moreover, it affects how hydrocarbon contaminants are distributed and degraded. (Kebede et al., 2021)

Microorganisms are typically highly adapted to a diverse range of salinities found in the earth oceans (Xua et al., 2010). There is no significant evidence indicating that microbes are impacted by a saline environment, such as saltwater. Salinity levels fluctuate periodically compared to the ocean, which is why it is crucial to determine if microbes introduced through bioaugmentation are compatible with the current saline levels in the atmosphere (Qin et al., 2012)

4. Phytoremediation

Phytoremediation is defined as the use of plants and the microbes that live with them to assimilate, transform, metabolize, detoxify, and degrade different hazardous inorganic and organic substances found in soil, water, groundwater, and air (Gkorezis et al., 2016). Phytoremediation began in the early 1980s as a research area with studies on the uptake of metals by hyper accumulating plants and the toxicity of pesticides to crops and no target plants (Simmer and Schnoor, 2022).

On the other hand, through the processes of water and mineral intake, root development, and related root exudates, plants may significantly alter the rhizosphere soil. The simplest root uptake model considers the uptake at the root surface as the first-order kinetics (Xie et al., 2024)

Phytoremediation is used in the field of remediation of various environmental contaminants, such as petroleum hydrocarbons, herbicides, explosives, and heavy metals (Baoune et al., 2018). Phytoremediation processes can be classified in to five groups (Vaziri et al., 2013)

4.1. Phytotransformation

The term phytotransformation represents a change in chemical structure without total compound breakdown. Phytotransformation is known as the "Green Liver Model", as plants act similarly to the human liver in dealing with these xenobiotic compounds (foreign compound or pollutant). Plant enzymes give xenobiotic more polarity after they are ingested by introducing functional groups like hydroxyl (Vaziri et al., 2013)**.**

The plant metabolic system employs the surrounding enzyme activities with the assistance of rhizosphere bacteria to reduce metal elements toxicity. Compared to other forms, phytotransformation is labor-intensive, often requires soil amendments, and is less reliable (Alsafran et al., 2022).

4.2. Phytostabilization

Involves using plants and the microbes that live with them as an ecologically friendly way to reduce the negative effects of pollution on the environment (Baoune et al., 2018). By specifically avoiding them toward surface and subsurface water, the plants will reduce pollution in the root zone (rhizofiltration) and erosion (especially caused by wind activity) (Laib., 2021).

4.3. Phytovolatilisation

It involves the diffusion of contaminants from the stem of the plant via its leaves to the atmosphere as a result of transpiration and plant metabolism. This procedure works well with volatile organic substances like trichloroethylene, benzene, toluene, ethylbenzene, and xylene (BTEX), naphthalene, and several inorganic compounds that have volatile forms like selenium, mercury, and arsenic (Ossai et al., 2019).

Heavy metals are absorbed through the roots and subsequently converted into a volatile condition, which is released into the atmosphere through the stomata. This approach acts as a temporary solution; the volatile metal is reintroduced into the soil through precipitation (Moreira et al., 2021). This technique does not permanently remove pollutants but rather transports and transfers them into the atmosphere from one medium to another, thus allowing the pollutant to be released into the soil from the atmosphere (Ossai et al., 2020).

4.4. Phytostimulation or rhizodegradation

Rhizodegradation is the increased biodegradation of resistant organic pollutants by bacteria and fungi linked to the roots under the influence of select plant species. Vegetation can increase the total number of beneficial fungi and bacteria in contaminated soil through a general rhizosphere effect. Furthermore, certain chemicals generated by roots possess the ability to activate genes for enzymes involved in the microbial degradation of recalcitrant organic contaminants (Azadeh et al., 2013). The rhizosphere's microbial activity serves to eliminate contamination. It is stimulated by racinary exudates secreted by the plant (Laib., 2021).

4.5. Phytoextraction

Phytoextraction, or phytoaccumulation, is a method using plants or algae to remove contaminants from soils, sediments, or water, with hyperaccumulators absorbing larger pollutants. It's become increasingly common in the last 20 years. Pollutants are absorbed by plants through their root systems, where they are either stored in the biomass of the roots or transferred to the stems and leaves. Pollutants can remain in living plants until they are harvested. To achieve significant cleanup, the technique usually needs to be repeated throughout numerous crop cycles because the amount of contaminants in the soil decreases after each harvest. Following cleaning, the soil can sustain further plants (Vaziri et al., 2013)

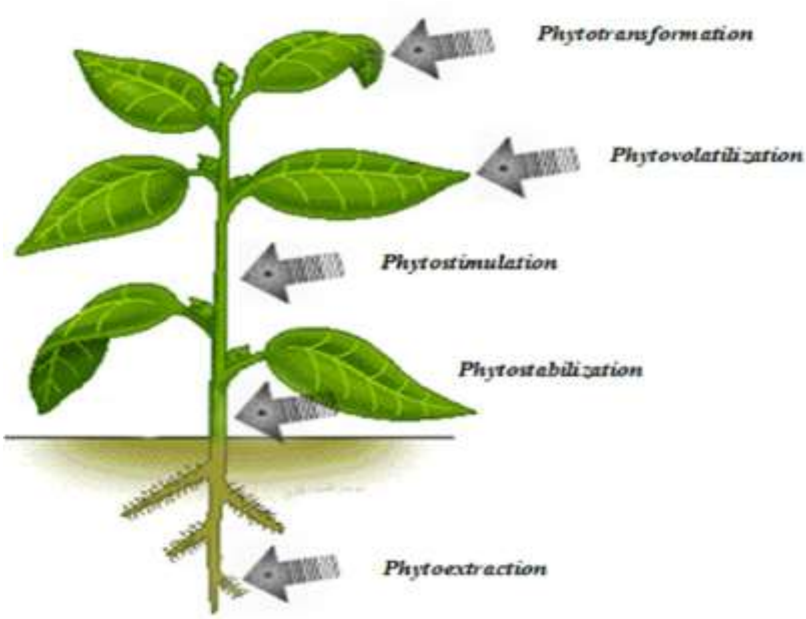


Figure. 1. Mechanisms of phytoremediation (Waoo et al., 2015).

Table 1. Examples of some plants used in phytoremediation.

Soil contaminants	**Plant species**	**Methods**	**Removal efficiency**	**References**
Crude petroleum oil	*Phragmites australis* and *Juncus Maritimus*	Attenuation, biostimulation in marshy Sediment	PHs removal efficiency achieved best with P. australis (16%)	Ribeiro et al. (2014)
Petroleum hydrocarbons in crude oil contaminated soil	wheat (*Triticum aestivum*), maize (*Zea mays*), white clover (Trifolium repens), alfalfa (*Medicago sativa*), and ryegrass (*Lolium multiflorum*)	soil amendments (biochar and compost) on plants belonging to Poaceae and Fabaceae families	The highest TPH removal (68.5%) by ryegrass with Compost, white clover with biochar (68%). Without any soil amendment, ryegrass and alfalfa showed 59.55 and 35.21% degradation of TPHs,	Yousaf et al. (2022)
Crude oil	Imperata cylindrica; Mucuna bracteata; Pteris vittata; and Epipremnum aureum;	Screening of plants in Malaysia at 5% crude oil contamination by weight	Imperata cylindrica (40%); Mucuna bracteata (31%), Pteris vittata (36%); Epipremnum aureum (50%); to remove total PHs within 42 d	Tang and Angela.(2019)

4. Interaction between plants and microorganisms

The interactions between inoculants, plants, and native organisms which include niches, predation, and competition for nutrients are crucial to the bioremediation process. These relationships are essential to the overall effectiveness of bioremediation (Rohrbacher and St-Arnaud, 2016).

The microorganism-plant combination method is the most popular technique for in situ remediation. Research shows that plant-associated microorganisms primarily metabolize organic contaminants during phytoremediation. Additionally, the remediation capacity of plants is influenced by the quantity of bacteria in their environment (Sui et al., 2021).

Fungi and bacteria can live inside plants; they can come from the rhizosphere, phyllosphere, and rhizoplane, among other places. Furthermore, the seeds have the ability to transmit these bacteria from one generation to the next. It is possible for both bacteria and fungi to live inside plants, either in the spaces between cells or within their own cells. This phenomenon results from a complex beneficial relationship between microbes and plants (Inge Jambon .2018).

Furthermore, root exudates are essential for promoting plant-microbe and microbe-microbe interactions that take place in the rhizosphere. Beneficial microbes, including nitrogen-fixing bacteria, mycorrhizal fungi, and plant growth-promoting rhizobacteria (PGPR), might be attracted to these exudates. It has been shown that certain compounds, such as sugars and amino acids, especially stimulate PGPR (Rohrbacher and St-Arnaud, 2016).

Certain organic acids released by plant roots, such as the intermediates of the citric acid cycle, have been linked to various processes, including the microbial breakdown of petroleum hydrocarbons in the soil. These organic acids have the ability to alter the chemistry of the rhizosphere, consequently influencing the availability of organic contaminants in the soil (Ite et al., 2019). The nature of the interaction between plants and soil microorganisms is determined by the type of root exudates. The first step in the colonization of plant roots is bacterial adhesion. Root colonization genes were studied using mutagenesis, with surface proteins, capsular polysaccharides, flagella, and chemotaxis playing roles in rhizobacteria's colonization abilities (Marihal and Jagadeesh., 2013).

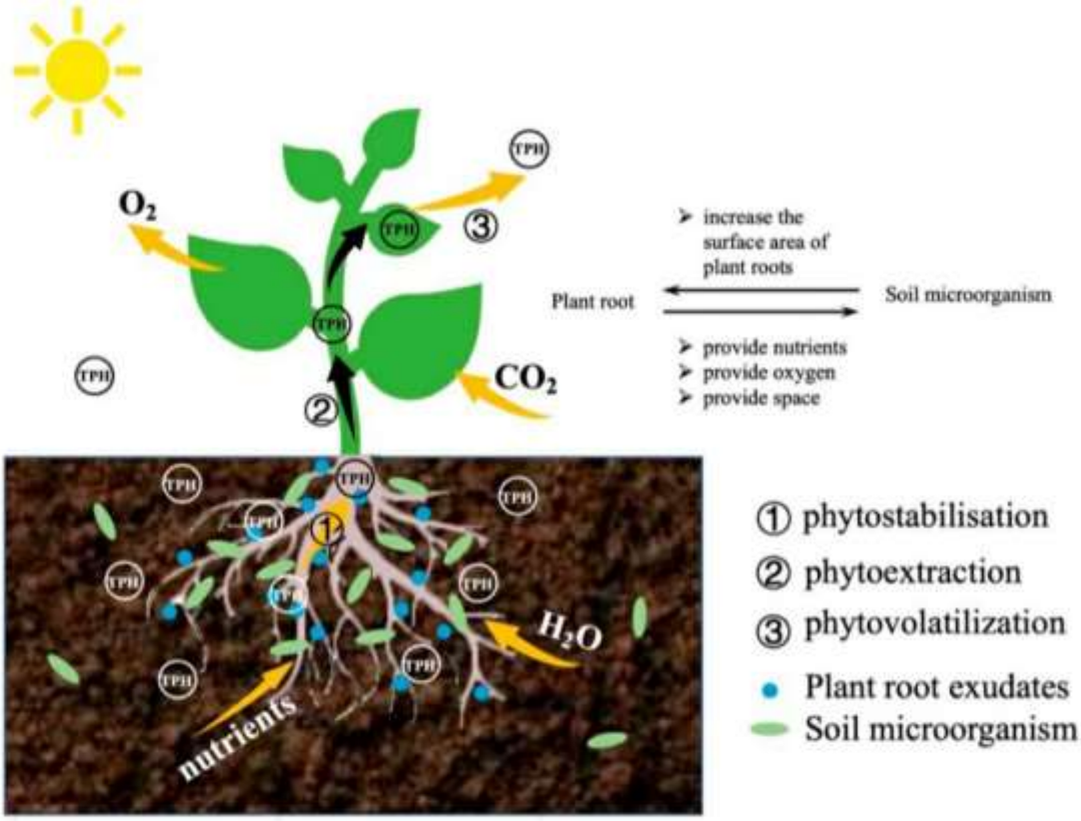


Figure 2. Interactions during pollutant remediation by microorganisms and plants (Sui et al., 2021).

Methodology

Methodology

1. Material

1.1. Bacterial strains

Five bacterial strains previously isolated from samples of petroleum products and from petroleum contaminated soils were assessed in this study. These were one Gram negative bacteria *Pseudomonas aeruginosa* and four Gram positive bacteria, *Enterococcus gallinarum, Aneurinibacillus migulanus*, *Streptomyces cinereoruber*, *Lysinibacillus cavernae*. Before use, their purity was checked by means of microscopic observations after Gram staining.

1.2. Soil sample

Soil sample was collected from Tiaret, Algeria

1.3. Diesel and gasoline oil

Gasoline and diesel samples were obtained from a gas station (Tiaret, Algeria).

1.4. Plant

Sunflower seeds (*Helianthus annus*) were delivered by the Technical Institute of Crops Improvement (ITGC), Sebaine, Tiaret (Algeria).

2. Methods

2.1. Preparation of soil

The first step in this study was the preparation of soil for the assay by subjecting it to a pre-treatment comprising air-drying followed by sieving to 1.5 mm (Carmine et al. 2020). After that, autoclaving it at 121ºC for 15 min three times (24 h apart) to remove the indigenous microorganisms. The soil was supplemented with 250 $mg.kg^{-1}$ of $(NH_2)_2SO_4$ and 100 mg.kg-1 of K_2HPO_4 to biostimulate the growth of microbial inocula (Bento et al. 2004). The sterilized soil was distributed over 101 pots where 42 pots were contaminated with 10 % diesel oil and the other 42 pots were contaminated with 10 % gasoline for bioremediation test.

2.2. Bioaugmentation test

Soils were inoculated with 3 ml volume of the standardized microbial suspensions (0.5 Mac Farland) of each strain alone and with microbial consortium, where the consortium was formulated by mixing equal proportions of pure bacterial cultures (Ghazali et al., 2004). After homogenization with a sterile spatula, the mixture is incubated for 7 days at room temperature. Sterile distilled water was added every 48 hours with homogenization to maintain the humidity and O_2 level in the medium in order to allow biodegradation of the pollutants. Triplicates are made for each microbial isolate, consortium and for the control (without microbial suspension).

Total petroleum hydrocarbons (TPH) degradation kinetics was followed by sampling at time intervals after 7 days, 14 days, 21 days and 28 days.

2.3. Planting seeds

The surface of seeds was disinfected by immersion in bleach for 5 minutes (Qu et al., 2011) to avoid the addition of non-native microorganisms to the system. Then the seeds were carefully rinsed three times with sterile water and left to germinate in the petri dishes 3 days. After germination, one seed was used for an experimental pot containing sterile soil contaminated by 10 % diesel and gasoline and inoculated with 3 ml of the bacterial suspension and the consortium separately. A control was used and consisted in soil containing one seed and inoculated with the tested microorganisms in addition to the consortium.

Figure 3. Seeds germination steps

2.4. Determination of total petroleum hydrocarbon (TPH) in soil

One gram from each soil pot was collected and placed in a 100 ml flask, then 2 ml of n-hexane was added. The mixture was agitated vigorously on a magnetic stirrer for 30 min to permit for hexane to extract the oil out of the soil sample. The solution was then filtered using a paper filter and 0.1 ml of the filtrate was diluted by adding 5 mL of hexane. The absorbance of the extract was measured spectrophotometrically at 400 nm using n-hexane as a blank. The TPH in the soil sample was calculated with respect to a standard curve obtained from testing several concentrations of diesel oil and gasoline (separately) diluted with n-hexane (Agarry and Latinwo., 2015).

Percent degradation (D) was calculated using the following formula:

$$D = \frac{TPHi - TPHr.100}{TPHi}$$

Where TPHi and TPHr are the initial and residual TPH concentrations, respectively

2.5. Statistical analysis

Results are expressed as means ± standard of deviation. All the experiments were replicated at least three times and quantitative data were subjected to analysis of variance. Comparison between groups was carried out using the test of Duncan. The significant differences between means were determined at $p < 0.05$ level.

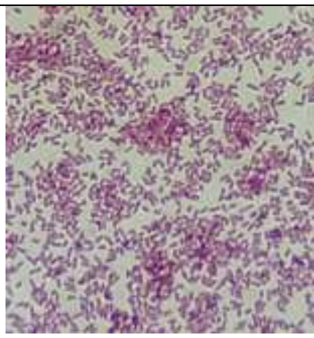
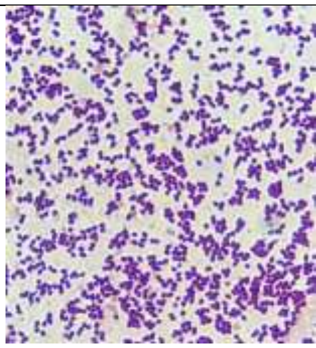
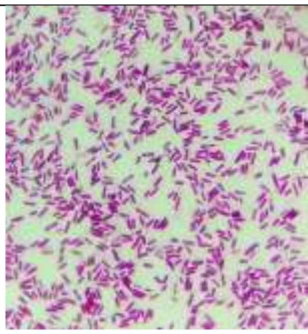
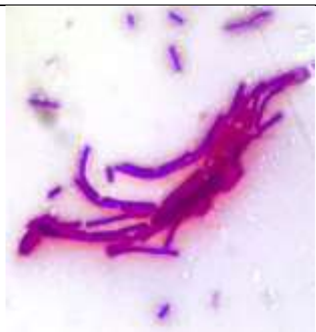
Results

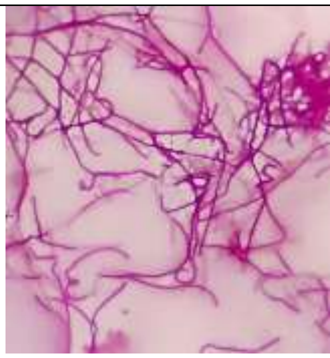
Results

1. Purity of the microbial isolates

Results of the macroscopic and microscopic observations of the microbial isolates are reported in table 3.

Table 2. Macroscopic and microscopic observations of the microbial isolates.

Bacteria	Macroscopic observation	Microscopic observation
Pseudomonas aeruginosa		
Enterococcus gallinarum		
Aneurinibacillus migulanus		
Streptomyces cinereoruber		

Lysinibacillus cavernae		

2. Degradation rates of diesel and gasoline oil in soil by bacterial strains and plant

After 21 days of incubation period of the bacterial isolates and the consortium, in soils contaminated with diesel oil, we observe that *Lysinibacillus cavernae* (59.78±19.21), *Enterococcus gallinarum* (57.06±11.52), consortium (54.51±2.71%), *Pseudomonas aeruginosa* (51.63±5.43), *Streptomyces cinereoruber* (50.72±3.13%), and *Aneurinibacillus migulanus* (48.91%) have demonstrated higher rates of degradation, with no statistically significant difference between them.

The strains *Enterococcus gallinarum*, *Aneurinibacillus migulanus*, and *Pseudomonas aeruginosa*) coupled with plants generally degrade diesel at a higher rate than their rates of degradation without plants as shown in fig.1, However, the strains *Streptomyces cinereoruber*, *Lysinibacillus cavernae*, and consortium showed the higher degradation rates without plants.

Pseudomonas aeruginosa (81.52±3.84%) and *Enterococcus gallinarum* (79.71±8.3%) had the highest percentage of degradation, with no significant difference between them .These were followed by *Aneurinibacillus migulanus* (67.93±3.84%), *Streptomyces cinereoruber* (47.55±5.76%). However, the consortium (38.04 ±4.70%) and *Lysinibacillus cavernae* (33.96±9.60%) showed the lower degradation rates.

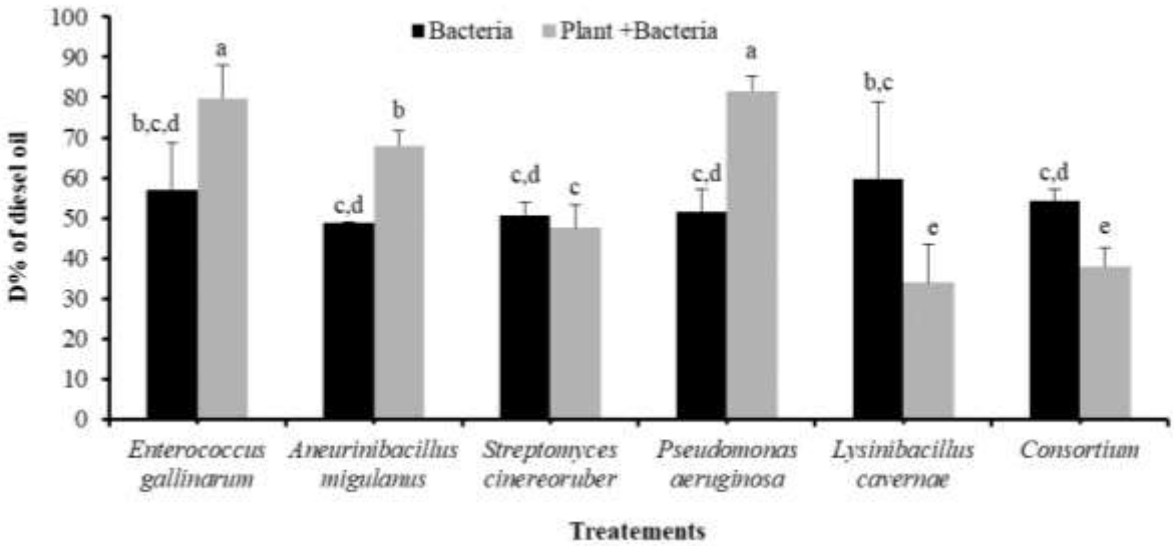


Figure 4. Percentage of degradation of diesel oil in soil.

Beside, regarding gasoline degradation by bacterial strains, we notice that *Enterococcus gallinarum* (68, 43±19.37%), demostrat the higher rate of degradation, folowed by *Pseudomonas aeruginosa* (57.05±16.14%), consortium (52.51±16.14%), and *Streptomyces cinereoruber* (43.37±16.14%). Furthermore, *Aneurinibacillus migulanus* (34.24±9.68%) and *Lysinibacillus cavernae* (34.24±9.68%), did not significantly differ in their rates of degradation.

However, the obtained result of degradation of gasoline by bacteria and plant showed globally that, the rates of degradation of all bacterial species coupled with plants are higher than their rate of degradation without plants, were the strains *Lysinibacillus cavernae* (89.8 ±5.27) and *Enterococcus gallinarum* (86.75±12.91), demostrated a higher rate of degradation with no significant difference between them, folowed by *Streptomyces cinereoruber* (76.10±2.63%), *Aneurinibacillus migulanus* (70.77±3.22 %).consortium (52.51±3.22), whereas *Pseudomonas aeruginosa* demonstrated a beter rate of degradation alone then with plant (57.05±16.14% 31.96±%), respectively

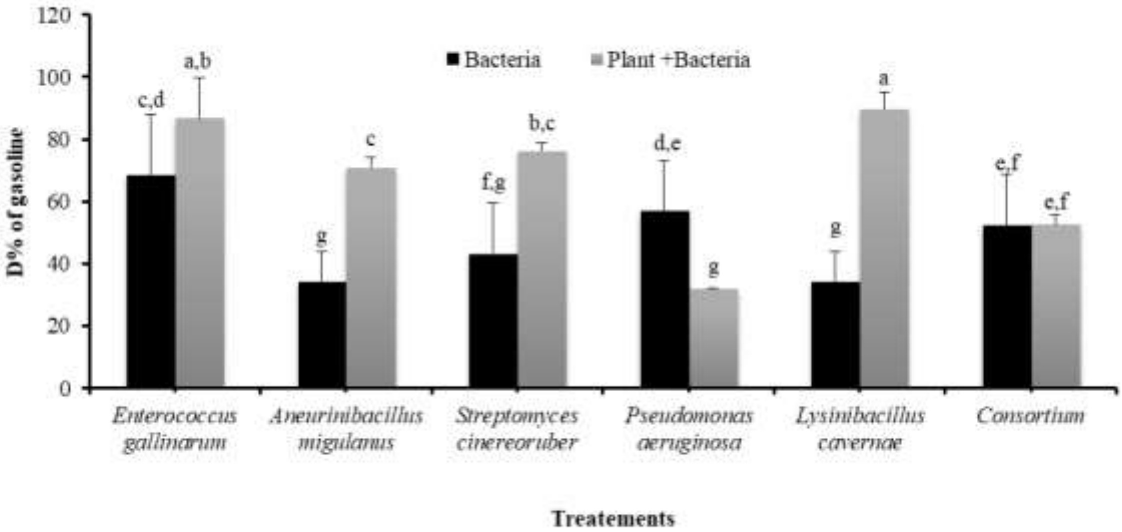


Figure 5. Percentage degradation of gasoline in soil.

3. Growth of plant

In this experiment, we observe that, the pots that contained sterile soil and were treated with bacterial strains *Streptomyces cinereoruber*, *Enterococcus gallinarum,* and consortium, and the pots that contained polluted soil treated with the same bacterial strains and consortium showed plant growth at differing levels, as seen in Fig. 6.

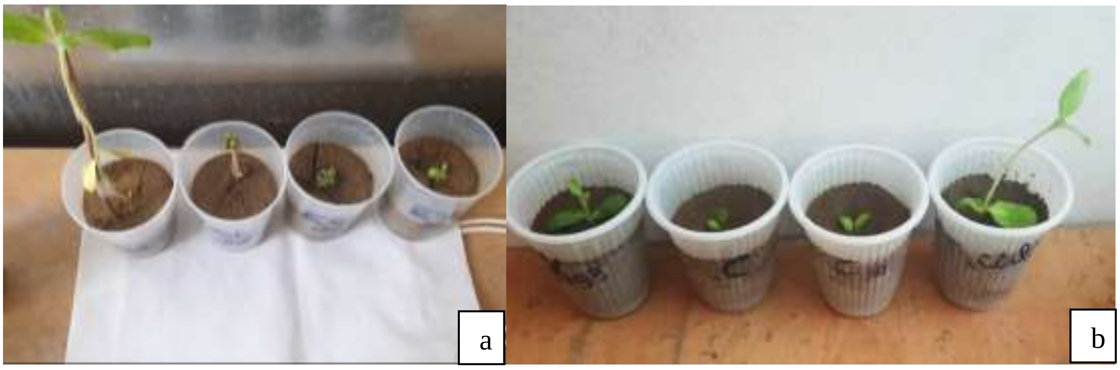


Figure 6. Plants growth in (a) polluted soil, (b) sterile soil)

After 21 days of experiments, different parts of plant were measured (table 3).

Table 3. Plant growth after 21 days.

	Negative Control	Positive control Gasoline	K33 (Gasoline)	C50 (Gasoline)	Consortium (Diesel)	K33	C50	Consortium
Leaves number	**8**	**2**	**5**	**2**	**2**	**6**	**2**	**6**
Root number	**11**		**12**	**24**	**4**	**9**	**8**	**6**
Root Length (mm)	**31.94**	**4.75**	**13.10**	**75.18**	**46.66**	**86.99**	**86.05**	**30.3**
Root Width (mm)	**0.14**	**0.47**	**0.33**	**0.36**	**0.55**	**0.11**	**0.14**	**0.08**
Stem Length (mm)	**250**	**11.78**	**40.04**	**0**	**2.22**	**41**	**0**	**37.58**
Stem Width (mm)	**1.35**	**3.27**	**0.44**	**0**	**0.51**	**2.94**	**0**	**1.16**

Discussion

Discussion

Petroleum hydrocarbons (PHs) are key energy sources and are used as raw materials in numerous industries. Nevertheless, PHs are harmful to organisms since they pollute the soil. To remediate contaminated soil, a variety of ex situ or in situ chemical and biological techniques are used (Ambaye et al., 2022)

The aim of this study was to test the ability of previously isolated microorganisms from samples of petroleum products and petroleum-contaminated soils, in combination with a plant to degrade gasoline and diesel oil in soil.

Five bacterial strains: one Gram-negative *Pseudomonas aeruginosa* and four Gram-positive *Enterococcus gallinarum, Aneurinibacillus migulanus, Streptomyces cinereoruber*, and *Lysinibacillus cavernae,* in combination with sunflower seeds *Helianthus annus* were tested in this study.

Microorganisms are adaptable little factories that, by using their own metabolic processes, may absorb, break down, and convert harmful substances like toluene, benzene, pyrene, etc. into harmless forms. Even though microbial bioremediation is economical and environmentally beneficial, it requires a long time and new technology to increase process efficiency (Waychal et al., 2022).

After 21 days of the incubation period of the bacterial isolates and the consortium in soils contaminated with either diesel oil or gasoline, all the tested strains and consortium demonstrated the ability to degrade both contaminants at different rates.

In this study, the rate of degradation of diesel oil by tested strains, such as *Pseudomonas aeroginosa*, *Enterococcus gallinarum, Streptomyces cinereoruber*, *Lysinibacillus cavernae,* and consortium, was generally more than 50%, and the higher percentage of degradation of gasoline was demonstrated by *Enterococcus gallinarum* (68.43%).

Pseudomonas aeruginosa has been successfully used to break down a variety of substances, including n-alkanes and PAHs, as well as gasoline, kerosene, diesel oil, and crude oil (Karamalidis et al., 2010).

Mixed cultivation shows a degradation advantage; this is assumed to be due to the synergistic effect between bacterial strains. In our experience, the degradation of diesel oil and gasoline by the consortium was 54.51% and 52.51%, respectively.

In addition, other studies used *Enterococcus* in consortiums for diesel and gasoline degradation of petroleum; for consortium 48%. The results of GC-MS analysis revealed that the removal of long-chain n-alkanes (C23–C32) was in the range of 35–58% (Ozyurek and bilkay.,2020).

Phytoremediation is a cost-effective and environmentally friendly method, offering a suitable alternative to chemical and physical approaches for the removal of pollutants from soil based on plants such as *Alhagi camelorum* (Nemati et al., 2024), *Helianthus annuus* (Rocha et al., 2019), and *Zea mays* (Baoune et al., 2019).

Plant-microbe interactions, together with organic soil amendments, offer an emerging trend for remediation of hydrocarbons. A study conducted by Hussain et al. (2018) found that soil amended with compost, biochar, and consortium (*Pseudomonas poae, Actinobacter bouvetii, Stenotrophomonas rhizophila* and *Pseudomonas rhizosphaerae*) showed the highest hydrocarbon removal (85%) in rehabilitating contaminated soil with Italian ryegrass and crude oil. In addition, Eze et al. (2022), examined the synergistic interactions of *Medicago sativa L.* and *Paraburkholderia tropica* WTPI1 for enhanced rhizoremediation of diesel fuel-contaminated soils. They found that combining *Medicago sativa L.* and *Paraburkholderia tropica* WTPI1 can significantly improve rhizoremediation of diesel fuel-contaminated soils, resulting in a 99% increase in plant biomass and the removal of 96% of diesel.

Phytoremediation and biodegradation of contaminants by plants and microorganisms may not achieve full remediation due to phytotoxicity and the evapotranspiration of pollutants (Supreeth, 2022).

In this study, after 21 days, the growth of germinated seeds in the soil contaminated with 10% diesel and gasoline does not exceed 40%. The inhibition of plant growth may be attributed to the presence of a toxic petroleum hydrocarbon compound (Rahbar et al., 2012).

Furthermore, the presence of hydrocarbons alters the characteristics of the soil, leading to a decrease in the availability of water and nutrients. The occurrence of drought conditions, induced by oil contamination, exerts stress on plants, affecting certain growth parameters. It is likely that the drought stress reduces the water potential of root and leaf cells to a lower level, which is essential for cell elongation, according to Agnello (2016).

Pollutant toxicity to native or introduced plants can lead to a variety of negative effects, including reduced growth of aerial parts and roots, reduced production of photosynthetic pigments, slowed nutrient absorption, and alteration of root architecture (Gkorezis et al., 2016)

On the other hand, the rate of degradation was accelerated by the coupling of microorganisms and plants. In our experience, the results of the degradation of diesel oil and gasoline with bacterial strains and plants showed that *Pseudomonas aeruginosa* and *Lysinibacillus cavernae* had the highest rates of degradation (81.52% and 89.8%, respectively). Nevertheless, the strain *pseudomonas aeroginosa* decreases its degradation rate of gasoline from 57.05% to 31.96%, compared to the consortium, which has no change in the results after 21 days with a degradation percentage of 52.51%.

In this experiment, we observe that, the pots that contained sterile soil and were treated with bacterial strains (K33) *Streptomyces cinereoruber*, (C50) *Enterococcus gallinarum*, and consortium), and the pots that contained polluted soil treated with the same bacterial strains and consortium showed plant growth at differing levels . This finding confirms the interaction between these microorganisms and plants that improves the remediation process. After 21 days we also observed that the number of roots of sunflower inoculated with bacteria strains was higher compared with non treated plant.

In plant-bacteria interactions, plants may be positively influenced by the presence of bacteria that are able to synthesize plant hormones; in turn, plants provide nutrients and residency for bacteria, which in turn can improve the applicability and efficiency of phytoremediation in sites contaminated by petroleum hydrocarbons (Gkorezis et al., 2016).

Other study, repport that the ability of the infected endophyte (*Pseudomonas sp.* J10) to produce the enzyme ACC deaminase may be the cause of the increase in plant biomass, root length, and shoot length. According to bacterial ACC deaminase activity, plants grow better when their roots expand and develop, which results in a larger root system, more root exudates, and a higher bacterial population in the rhizosphere (Iqbal.,2019).

The polluted soil employed in this study appeared to be a toxic environment for sunflour plants, leading to substantial deleterious effects on sunflour germination and growth. After 15 days, early plant death was seen, and mortality rates are continual.

In another investigation, the amount of gasoline removed from soil when sunflower was present decreased from 54.06% to 49.42% at 500 mg/kg. This indicates that an increase in the initial gasoline content of the soil from 500 to 5000 mg/kg resulted in a maximum 8.6% reduction in the effectiveness of sunflower phytoremediation (Zand and Hassan.,2016).

Results of the current research and our experience indicate that the gasoline content of soil cannot be considered a controlling factor affecting the phytoremediation potential of sunflowers when the soil is contaminated with low to moderate levels of gasoline and diesel.

The identification of microorganisms capable of promoting both plant growth and hydrocarbon degradation is crucial to the success of plant-based remediation techniques. In addition, plants need to demonstrate certain traits in order to live and thrive in petroleum hydrocarbon-contaminated environments. These traits include resistance to one or more petroleum mixture components, high competitiveness, fast growth, and the capacity to synthesize and release hydrocarbon-degrading enzymes (Gkorezis., 2016).

Conclusion

Conclusion

The rapid economic growth in countries worldwide and increased human activities impacting vegetation have resulted in the release of numerous persistent pollutants into water, soil, and air. Current concentrations of these pollutants exceed acceptable thresholds, leading to severe health issues for both humans and other organisms in the environment. The success of biological methods for removing pollutants from contaminated sites hinges on the interaction between suitable microorganisms and plants.

Plants and microbes have long played pivotal roles in bioremediating soils contaminated with petroleum hydrocarbons, offering an alternative to traditional physico-chemical methods for restoring polluted sites. Utilizing a combined system of microorganisms and plants for pollutant removal offers several advantages over other approaches. Firstly, it is cost-effective, environmentally friendly, and manageable. Secondly, it enhances green cover and provides habitat for numerous organisms within the biosphere.

In our study, we evaluated the degradation process of petroleum hydrocarbons by integrating five bacterial strains (*Pseudomonas aeruginosa, Enterococcus gallinarum, Aneurinibacillus migulanus, Streptomyces cinereoruber,* and *Lysinibacillus cavernae*) with sunflower seeds (*Helianthus annuus*). The study conclusively demonstrated that integrating plants with bacterial strains significantly enhances degradation rates of petroleum hydrocarbons. Notably, *Pseudomonas aeruginosa* and *Lysinibacillus cavernae* exhibited the highest degradation rates for diesel and gasoline, respectively. Plant growth was observed in both sterile and polluted soils treated with *Streptomyces cinereoruber*, *Enterococcus gallinarum*, and the bacterial consortium, confirming that combining plants with bacteria improves the remediation process.

A deeper understanding of plant-microbe interactions can be leveraged to further develop phytotechnologies for site cleanup and apply bioremediation strategies to petroleum hydrocarbons contaminated soil through a series of metabolic transformations, biological, chemical, and physical processes.

For practical application of microorganism-plant combined systems in remediating contamination at an industrial scale, conducting scale-up studies before commercialization is essential. Another significant challenge in this field is the bioaccumulation of pollutants by plants and their disposal after the remediation process, which impacts the food chain and the environment. Preferably, ornamental plants that are not consumed by humans or animals should be used, as these plants absorb pollutants from the soil and store them in their roots or tissues. This could potentially lead to the accumulation of pollutants in the food chain if consumed. Moreover, ornamental plants enhance the landscape without posing risks to public health.

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