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Rajendra Pavan
Research Scholar, Department of Zoology, Himalayan University, Itanagar, Arunachal Pradesh, India

Brijesh Sharma
Associate Professor, Department of Zoology, Himalayan University, Itanagar, Arunachal Pradesh, India

Correspondence
Rajendra Pavan
Research Scholar, Department of Zoology, Himalayan University, Itanagar, Arunachal Pradesh, India

Morphological and biochemical characterization of oil degrading isolated microorganisms

Rajendra Pavan and Brijesh Sharma

Abstract
Environmental microbiology's most crucial component is the effective bioremediation of dangerous and deadly contaminants. The biological procedure known as "bioremediation" uses living microorganisms to eliminate pollutants or toxins from soil or water. Microbes are used to detoxify and degrade environmental pollutants in this process. Toxic hydrocarbons are converted into innocuous compounds including organic acids, aldehydes, and eventually carbon dioxide and water by this process. The ecology is impacted by oil contamination, which in turn influences the bioremediation procedures. Different hydrocarbon groups in a complex mixture have varying bioavailability and biodegradability, which results in varying intrinsic bioremediation application. Therefore, before conducting remedial action, a site assessment should determine the hydrocarbon composition based on the source of the pollution. This study's site assessment yields a number of broad conclusions that might be used to build degradation strategies. In comparison to non-contaminated soil, contaminated locations have a relatively high concentration of microorganisms that break down hydrocarbons. However, the degrader numbers differ between locations and most likely change in response to the length of pollutant exposure. According to the findings of the current study, soil samples contaminated with petroleum were taken from a variety of oil-affected locations. The physical and chemical characteristics of the soil samples were ascertained. There was a direct correlation between contamination levels and carbon content. Additionally, it was discovered that the soil samples were lacking in phosphorus and nitrogen. The moisture content and water retention capacity of soil samples were low while the electric conductivity was high. The altered physicochemical characteristics of the contaminated soils render them unsuitable for the development of agricultural crops as well as the typical soil flora. By using the enrichment culture technique in selective minimum media (Bushnell-Hass) with the 2T engine as the only source of carbon, a total of eleven bacterial isolates and one fungus isolate were obtained.

Keywords: Bioremediation technologies, microorganisms, soil contaminated

1. Introduction

One of the most significant environmental concerns now recognized is oil contamination. It might endanger the ecosystem. Environmentalists feel quite threatened by it, and if it gets out of hand, it is very challenging to control. Both land and the ocean can become contaminated with oil. Enzymes secreted by microorganisms are in charge of breaking down the hydrocarbons found in petroleum. Such enzymes prevent biodegradation when they are absent. The biodegradation of petroleum hydrocarbons involves a wide variety of enzymes and metabolic pathways. The microorganisms utilized in the bioremediation process are either naturally occurring at a contaminated site or have been isolated from a different location and added to the contaminated site. Being nature's original recyclers, microorganisms, especially bacteria and fungi, are used to break down petroleum hydrocarbons. The mixed culture is typically preferred in the bioremediation process because it provides synergistic interactions over pure culture. Many microbes are capable of destroying soil-borne petroleum hydrocarbons. Ex situ and in situ bioremediation technologies can be broadly categorized. With the ex situ approach, the hazardous or waste can be removed from the polluted area, and the needed microorganisms can be used to perform bioremediation at designated locations. Even though the process is quite expensive, there may be a problem with disposal once it is finished, and the polluted areas may also be in very bad shape. The in-site treatment of pollutants is a component of in situ bioremediation technology. In comparison to physical and chemical treatment, in situ technology offers a variety of benefits. It permits cost-effective remediation of a polluted location without causing any inconvenience.

Comparing the cost-effectiveness of bioremediation to some physicochemical procedures, which are exclusive and necessitate regular supervision to achieve successful results, is one of the research of bioremediation's major advantages in the treatment of crude oil polluted soil. Because to its versatility and adaptability in diverse settings, bioremediation has become a promising technology for environmental treatment for organic substances, such as petroleum hydrocarbon. By using biological agents, complex or simple chemical compounds are converted into nonhazardous forms, resulting in materials with a higher nutritional value or simply reducing the final bulk of the product.

A developing method for treating soil contaminated with petroleum hydrocarbons is bioremediation. The process of removing toxic waste from the environment or converting it into non-toxic compounds under regulated circumstances has been dubbed "bioremediation," and it makes use of the biodegradative capability of biological agents, specifically microbes. The process of adding materials to a contaminated site or creating suitable conditions to hasten the natural biodegradation process is known as bioremediation. The function of bioremediation is based on biodegradation, which results in complete mineralization of complex organic contaminants into the simpler form, namely water, carbon dioxide, and cell protein by the action of microorganisms. In contrast, the conventional methods (adsorption, vapourization, and extraction) have a negative impact on the environment and transfer the pollutant from one environment to another, such as soil to atmosphere. Also, it is thought that bioremediation technology is the most dependable, non-intrusive, and likely least expensive alternative for treating some chemical contamination. Protecting soil quality and enhancing the timely breakdown, transformation, remediation, or detoxification of contaminants by biological methods are the primary goals of soil bioremediation.

Biostimulation and bioaugmentation are two of the key bioremediation technology methods. The procedure of biostimulation involves adding nutrients to the local microorganisms already present at the contaminated site in order to improve their growth and metabolic capacity for the breakdown of pollutants. Since the rate of decomposition of these native microbes is sluggish, additional nutrients are supplied to promote their activity. The bioremediation process is accelerated by the addition of nutrients since they boost the population of local microorganisms. When the local microorganisms are unable to remediate the contaminated site, bioaugmentation is the practice of introducing specific microbes or genetically modified microbes for the degradation of specific contaminants to speed up the transformation and biodegradation process.

Deep understanding of the characteristics or environmental conditions that affect the microbial biodegradation of petroleum hydrocarbons is necessary for the successful implementation of bioremediation technology in the petroleum hydrocarbon-contaminated site. Physical and biological factors both influence the biodegradation of petroleum hydrocarbons. Temperature, pH, nutrition, moisture content, hydrocarbon composition or molecular weight, pressure, bioavailability, salinity, etc. are examples of physical factors. By creating a perfect habitat with the right physical elements, bioremediation can be completed more quickly. The biological factors include the bacteria, fungi, algae, and protozoa that are necessary for the breakdown of petroleum hydrocarbons. Under ideal environmental conditions, the selection of the proper microbe is a crucial element in the breakdown of petroleum hydrocarbons at the polluted site.

Technology for bioremediation can be used in both in situ and ex situ circumstances. Without removing the soil, in situ bioremediation treats contaminants in the same location as the contaminated site (matrix). Examples of in-situ bioremediation include land treatment, biosparging, and venting. Ex situ bioremediation is the process of treating contaminants by excavating the soil (or "matrix") from the contaminated location. Land farming, composting, biopiles, and other methods are examples of ex situ bioremediation. Recombination DNA Technology (RDT), which creates genetically engineered microorganisms, is a biotechnological feature that considerably benefits bioremediation technology (GEMs). The rate of biodegradation of petroleum hydrocarbon pollutants in the environment is accelerated using either naturally occurring or genetically created bacteria as a seed. Recent developments in molecular biology techniques, such as the separation of plasmid DNA and the creation of DNA probes, are used to analyse the breakdown of petroleum hydrocarbons by the microbial community.

1.1 Principles of bioremediation

A complex mixture of hydrocarbons made up of thousands of distinct chemical components makes up crude oil. Due to the fact that each type of oil has a particular composition, there are several methods for treating oil using microorganisms and flora. Environmental pollutants are biologically digested by microorganisms into less dangerous forms during the bioremediation process, which takes place in controlled environments. Applying garden fertilizer to an oil-contaminated site is a simple example of a bioremediation strategy. A more intricate example is an engineered treatment "cell," which involves manipulating, aerating, heating, and treating soils or other media with various chemical compounds to promote biodegradation. Bioremediation can happen spontaneously or can be accelerated by adding bacteria and fertilizers. The type and pace of contamination as well as the unique characteristics of the polluted site influence the decision of the bioremediation technique. The oil's constituents' volatility, solubility, and susceptibility to deterioration vary. Certain oil molecules are readily biodegradable, whereas others resist biodegradation. The bacteria first identify the oil and its constituent parts before using the hydrocarbon present in the petroleum as a source of carbon and energy. Although the different petroleum hydrocarbon molecules are biodegraded at various rates by various microbe species, it happens simultaneously. Hence, as time passed, each component of petroleum gradually disappeared (ISU Engineering Information Technology).

Nutrient addition at the contaminated site is necessary to promote the growth, activity, and population of microorganisms because there is a chance that there won't be as many as are necessary for the bioremediation process. For hydrocarbon remediation, the proportions of carbon, nitrogen, and phosphorus needed are 100:10:4. (C: N:P). Both the early breakdown of hydrocarbons and microbial decomposition of hydrocarbons require oxygen. When oxygen is present, oil completely deteriorates. Under anaerobic circumstances, breakdown of petroleum hydrocarbons also took place when nitrate, iron, or sulphate were utilized as electron acceptors. Anaerobic degradation requires more time and results in a slower rate of deterioration. Anaerobic circumstances allow for the easier degradation of otherwise resistant molecules than the aerobic conditions needed for bioremediation. Environmental factors like pH, temperature, moisture content, and others have an impact on how quickly petroleum hydrocarbons degrade. The best environmental conditions must be created for the bioremediation process to be successful since environmental factors influence microbial growth and activity. The following categories can be used to categories microorganisms.

Degradation occurs aerobically when oxygen is present. This group of microbes is known to exclusively use pesticides and petroleum hydrocarbons (both alkanes and polyaromatic chemicals) for their carbon and energy needs. Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, and Mycobacterium are among the bacteria in this group, which are grouped according to their capacity for degradation.

1.2 Strategies for cleaning up pollution from crude oil

Only 10 to 15% of crude oil that has been spilled over into marine water may be recovered using physical and chemical procedures. When using the right microorganisms, highly hazardous oily material can be easily mineralized to safe end products at a very low cast rate, making bioremediation thought to be a self-driven, economical, and environmentally friendly method in comparison to physical and chemical methods like booms, skimmers, adsorbents, chemical surfactants, oxidants, etc.

Researchers have been putting a lot of effort into finding an effective and efficient approach to eliminate the toxins from oil from the environment in recent years. The process of biodegradation can be defined as the transformation of chemical molecules into energy, cell mass, carbon dioxide, and biological waste products by living microorganisms. There are some drawbacks to conventional activated sludge treatment, including low resistance to loading rate, sludge expansion, sensitivity to low temperatures and toxic compounds, loss of activity, biomass, and the need for additional equipment for accumulating sludge, instability due to loading shock and fluctuation, and additional treatment of excess sludge. The biological approaches preserve the quality of the environment (soil or water) during the remediation process, making them environmentally benign. Also, these solutions are less expensive than cleanup techniques that involve physical and chemical means.

1.3 Bioremediation of soil contaminated with crude oil

The majority of settings, where hydrocarbons may act as sources of organic carbon, harbour the microbes that degrade hydrocarbons. Bioremediation, which has various advantages over traditional chemical and physical treatments, is based on the employment of microorganisms or microbial processes to breakdown environmental toxins. It might be an economical and eco-friendly technology. Biodegradation is the physiologically mediated decrease in chemical compound complexity.

Because fungi can quickly ingest into the soil matrix, it would be intriguing to develop the bioremediation process employing fungi. Moreover, they may thrive in conditions with low humidity, an acidic pH, and low nutrient concentrations. There have been several different bioremediation methods created, but biostimulation is the most popular one. In order to remove contaminants effectively, native soil microorganisms must be activated by the addition of nutrients that this required 1x103 CFU g-1 of soil; however, other factors to be taken into account include the molecular structure and bioavailability of the contaminants). The microbial culture must be able to resist various soil environmental conditions and be viable when other microorganisms are present. Biosurfactants work by emulsifying hydrocarbons to increase crude oil solubilization and make it more accessible for microbial decomposition.

2. Results and Discussion

Microbial isolates viewing growth on Bushnell Hass agar plates were used for morphological and biochemical characterization.

2.1 Morphological Characterization of oil degrading isolates

2.1.1 Characterization of culture

Petroleum hydrocarbon degrading microbial isolates were characterized on the basis of their size, shape, opacity, and pigmentation. Morphological Characteristics of all 12 isolate are shown in Table-1. According to Table 4.6 out of twelve isolates seven isolates were shown in circular shape with smooth and rough texture, yellow and white in pigmentation.

Table 1: Characterization of microbial isolates isolated from oil contaminated soil samples

S. No.	Isolate	Shape	Texture	Opacity	Elevation	Pigmentation
1	PSB-I	Circular	Shiny	Opaque	Flat	White
2	PSB-II	Circular	Smooth	Opaque	Raised	Yellow- green
3	PSC-I	Rhizoid	Rough	Translucent	Convex	White
4	PSC-II	Circular	Smooth	Opaque	Convex	Light -Orange
5	PSC-III	Circular	Smooth	Opaque	Raised	Yellow
6	PSD-I	Circular	Shiny	Opaque	Convex	White
7	PSD-II	Irregular	Rough	Opaque	Flat	Yellow
8	PSD-III	Circular	Smooth	Translucent	Convex	White
9	PSE-I	Irregular	Rough	Translucent	Convex	White
10	PSE-II	Irregular	Rough	Opaque	Raised	Light- Orange
11	PSF-I	circular	smooth	opaque	Raised	Yellow
12	PSF-II	filamentous	smooth	opaque	Convex	White

Gram's Staining

According to Table -2 All the oil degrading isolates shows gram- negative results except PSC-Iwhich shows gram-positive result. 8 isolates were shown in cocci shape and another isolates shown rod bacillus and rod shape. In color according to Table mostly all isolates shown in pink color.

Table 2: Gram`s staining results of oil degrading isolates

S. No.	Sample	Morphology	Colour
1	PSB-I	Cocci	Pink
2	PSB-II	Cocci	Pink
3	PSC-I	Rod	Purple
4	PSC-II	Cocci	Pink/White
5	PSC-III	Cocci	Pink/White
6	PSD-I	Cocci	Pink/White
7	PSD-II	Cocci	Pink
8	PSD-III	Rod Bacilli	Pink
9	PSE-I	Cocci	Pink/White
10	PSE-II	Rod Bacilli	Pink/White
11	PSF-I	Cocci	Pink
12	PSF-II	Rod	Pink

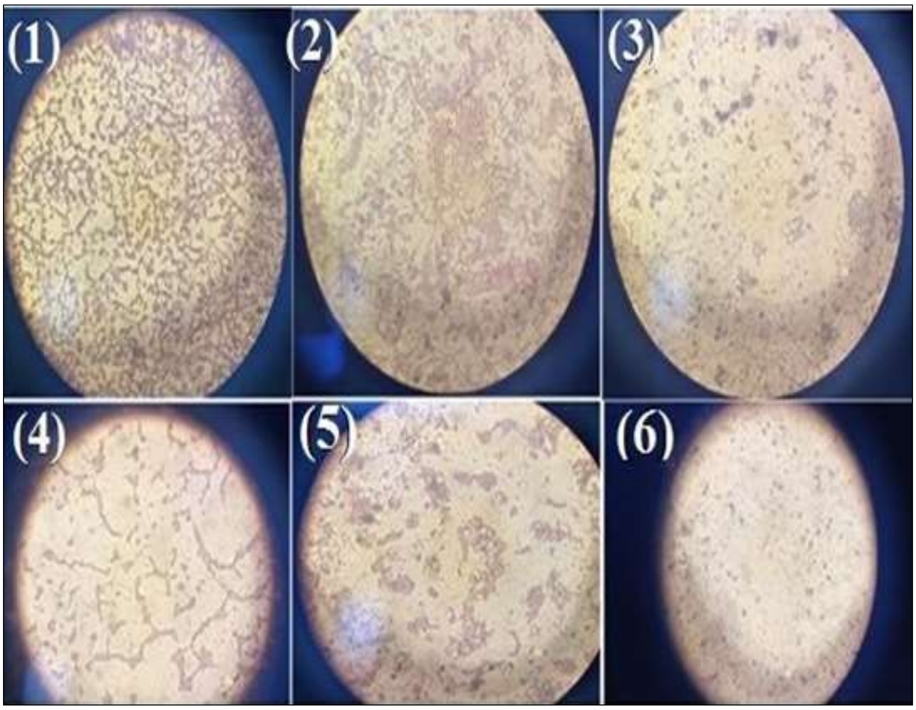

Fig 1: Gram`s staining results of oil degrading isolates

2.2 Biochemical characterization of petroleum hydrocarbon degrading microbial isolates

Different biochemical tests were performed for isolated oil degrading microbes. According to Table -3 the microbial isolates were shown positive results for oxidase test, catalase test. Casein hydrolysis, starch hydrolysis and indole test were recorded positive for only one isolates and another isolates were shown negative for these test.

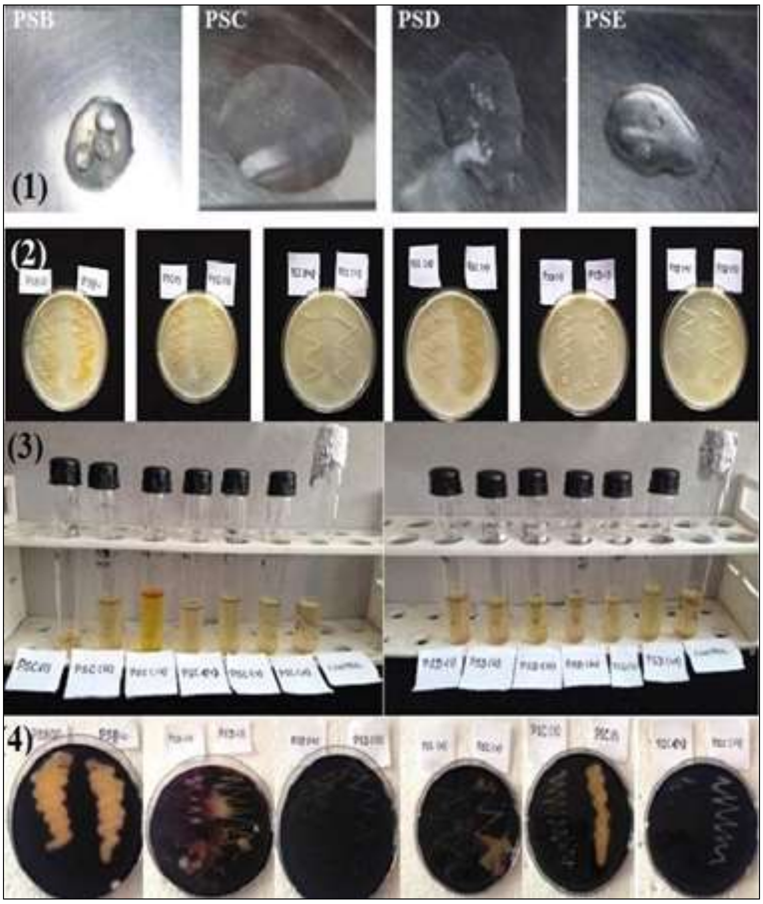

Fig 2: Biochemical characterization test (1) catalase test (2) casein hydrolysis (3) citrate hydrolysis (4) Indole production

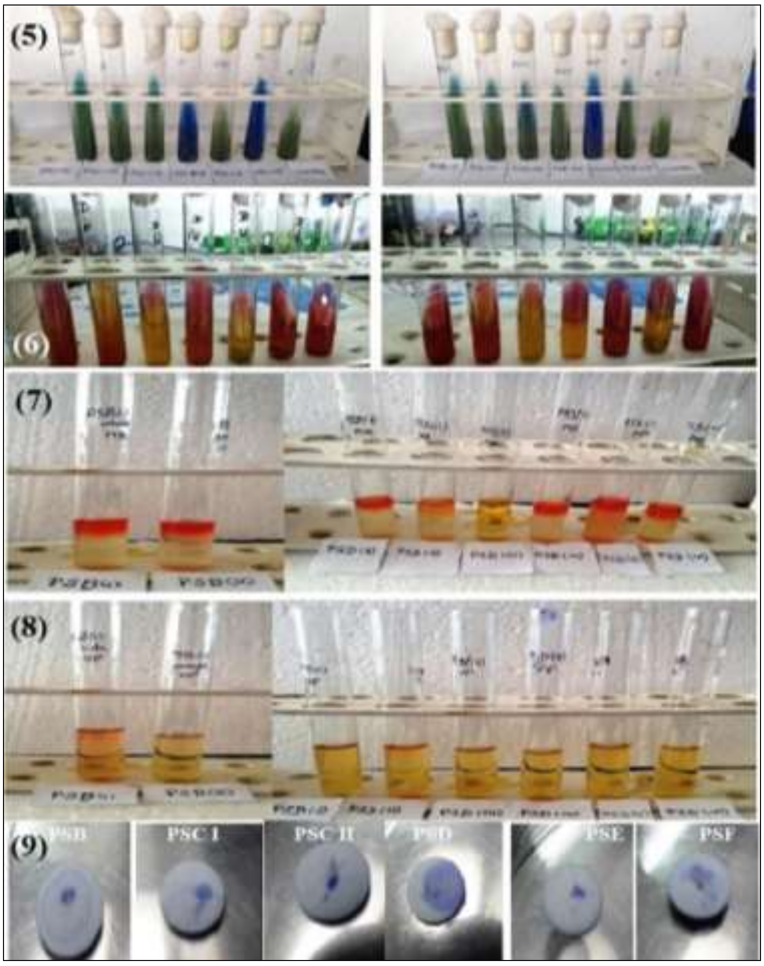

Fig 3: Biochemical characterization test (5) starch hydrolysis (6) Kligler agar test (7) Mr reaction (8) vp reaction (9) oxidase test

Table 3: Biochemical characteristics of oil degrading isolates

S. No.	Sample	NI1	NI2	NI3	NI4	NI5	NI6	NI7	NI8	NI9	NI10	NI11	NI12
	Biochemical test												
1.	Lactose utilization	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve
2.	Glucose utilization	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
3.	Gram`s reaction	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4.	Indole test	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5.	Motility	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
6.	Catalase test	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-	+ve	-ve	-ve
7.	Methyl red test	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
8.	VP Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9.	Oxidase test	+ve	-ve	ve +	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
10.	KIA	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
11.	Casein Hydrolysis	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
12.	Starch Hydrolysis	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
13.	CTT	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve

*(+) = Positive reaction, (-) = Negative Reaction, VP: Voges Proskauertest, KIA: Kligler iron agar, CTT: Citrate utilization.

3. Conclusion

The importance of life on Earth is strongly correlated with the general state of the environment. Our land and natural resources were treasured in earlier times as the boundless wealth it was intended to be. Nowadays, the catastrophic devastation of the ecology is caused by an expanding number of companies. In many nations around the world, the issue of land contamination is becoming more and more important. The initial step in the creation of a bioremediation method is the isolation and screening of effective pollution degraders. Screening is the process of using highly selective techniques to isolate only the microorganisms of interest from a huge population of microbes. Therefore, for screening to be efficient, it must permit the easy selection of the tiny percentage of valuable microbes while also permitting the dumping of a large number of worthless microorganisms in one or a few steps. The obvious place to seek for such cultures is polluted soil. Therefore, soil samples were taken from petroleum-contaminated sites in order to isolate PHC degraders.

To ascertain the physical elements, nutrients, and contaminants that could indicate the kinds of microorganisms recovered from the soils, the physico-chemical characteristics of the soil samples were conducted. Multiple heavy elements, including lead, zinc, cadmium, iron, and nickel, highlighted the soil samples' contamination. PHC pollution has negative effects on soil conditions, microorganisms, and plants. It also causes the loss of organic matter content, deterioration of soil structure, and loss of soil minerals like potassium, sodium, calcium, magnesium, nitrogen, and sulphate, phosphate, and nitrate.

Because some factors affecting the degradation process, such as movement of nutrients through soil pores, soil aeration, water holding capacity, and many others, are also under the direct or indirect influence of soil properties, physical properties of soil, such as bulk density and soil texture, have also been considered to be very essential for bioremediation. One of the most crucial physical characteristics of soils is its texture. It is determined by the relative amounts of the three different types of soil particles (soil separates) or mineral components of soil, sand, silt, and clay. The physical, chemical, and behavioral properties of soil are greatly influenced by its texture, particularly its ability to retain nutrients and moisture (Brown, 2003). In the current study, PCS-2 with a high clay content (33.9%) demonstrated less hydrocarbon degradation, while PCS-1 and PCS-3 with low clay and sandy loam (24.6 and 25.1%, respectively) demonstrated the maximum deterioration of 2T engine oil. Clay concentration was found to be 33.9% in PCS-2, which directly affects moisture content and water holding capacity, which in turn affect microbial development and the biodegradation of petroleum chemicals.

An ideal pH range is necessary for bioremediation to be effective. In exchange, the pH level of the soil might fluctuate due to the metabolites accumulated during breakdown and CO_2 emitted by microbial respiration. Oxidation of oil during biodegradation increases acidity. In the current investigation, the pH of both contaminated and uncontaminated soil samples is within the range of 7.00 to 8.00., the pH range of 6.5 to 8.5 is ideal for hydrocarbon breakdown. Additionally, observed that the pH range of 5.0 to 7.8 is ideal for the mineralization of oily sludge in soils.

3.1 Conflict of Interest

Not available

3.2 Financial Support

Not available

4. References

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