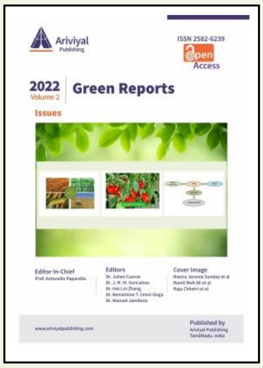
Green Reports

Ariviyal Publishing

Review Article

DOI: 10.36686/Ariviyal.GR.2022.03.07.033

Green Rep., 2022, 3(7), 16-24.



Environmental Phytoremediation Study of Oil Spill Site Using Common Vegetables: A Review

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ISSN: 2582-6239

Crossref

Publication details
Received: 15th December 2021
Revised: 28th February 2022
Accepted: 28th February 2022
Published: 10th March 2022

Abstract: The remediation procedure of debased and polluted soils, groundwater, and surface water by substantial metals needs a few strategies to expel the metals from debased zones. A few strategies have been utilized for expelling the toxins from the polluted conditions. Soils that are polluted with substantial metals can be treated by phyto-mining, corrosive draining, soil washing, physical or on the other hand mechanical partition of the contaminant, electro-compound treatment, electro kinetics, synthetic treatment, warm or pyrometalurgical partition and biochemical procedures. Phytoremediation is the utilization of plants to expel contaminants from soil and water. It is a moderate yet ecologically inviting approach to evacuate poisons. Plant materials, for example, organisms, lichens, tree covering, tree rings and leaves of higher plants have been utilized for a long time to identify the statement, aggregation and circulation of substantial metals contaminations. Lower plants particularly greenery and lichens have been generally utilized because of their ability for metal collection. Some higher plants have likewise been utilized for bio monitoring of substantial metals.

Keywords: environmental pollutions; oil spillages; remediation; vegetables; absorption

1. Introduction

Environmental contamination and pollution is a global problem for man and animal and has become a major public and environmental concern. Environmental contamination causes several human effects which could be fatal and it has been a major source of human health risk throughout the world. Heavy metals and crude oil contamination are some of the major environmental pollutions which are of public concern. Their health effects have been in existence over time, exposure to crude oil contamination and heavy metals have been on rise at different parts of the world. Development of modern technology and Industrial evolution leading to pollution caused by crude oil and heavy metal contamination are found within the areas of increased industrial activities. The environmental pollutants are spread through diverse routes, many of which finally find their way into food chain of livestock and man. Scientific reports have documented adverse impact of pollution on domestic and wild animals in the form of specific chemical behavioural changes, toxicities and population decline.[1-8]

Petroleum is a global consistent demanded product and subsequently oil spill is experienced during petroleum prospecting, investigation, extraction, exploration, transportation and use.[18-20] A portion of the major notable oil slicks incorporate the disaster area of Torrey Canyon of the expense of England in 1967, Exxon Valdez spill in Prince William South Alaska, United States in 1989, as of late the Shell spill Bonga field of Nigeria in 2011 and even most as of late the MV Marathassa spill, British Columbia, Vancouver, Canada in 2015. The biggest oil spill in history was BP's Deep water Horizon oil-boring stage blast which slaughtered 11 laborers and spilled 210 million US gallons on April 20, 2010. Reasons for oil slick incorporate normal causes, gear disappointment, human mistake, and blow out among others. Oil slicks decimate the biological system and brought harmful substances into nature. Oil is made out of different extents of hydrocarbon, for example, alkanes, aromatics, and polycyclic aromatic hydrocarbons (PAHs) and non-hydrocarbon including compounds containing sulfur, nitrogen, oxygen and metals.[4-12]

In a petroleum compound, metals do not exist in a free state but they exist as ligands (organometallics) and normally distil at high temperatures. Most heavy metals ranging from Molybdenum with atomic number 42 have been recorded in petroleum. Virtually in all crude oil, Vanadium and Nickel are present and they exist at higher concentration than other heavy metals found in petroleum.[6,19] Phytoremediation is an ecosystem plant based remediation technology which is engineered through the use of green plants for in-situ treatment of contaminated soil. It is an environmentally friendly process which takes advantage of the unique and selective uptake abilities of plant roots, in addition to the translocation, bioaccumulation, and degradation abilities of the entire plant body.[9-18]

Phytoremediation is cost effective and aesthetically pleasing because the plants can be easily monitored and metals absorbed by the plants may be extracted from harvested plant biomass and then recycled. It helps to conserve the environment, it combats the spreading of contaminants in air and soil, it is suited to remediate a very large area of soil, and the process is amenable to a variety of inorganic and organic compounds. However, a major con of phytoremediation is that it is limited to the surface area and depth occupied by the roots. It requires long-term commitment, because the process depends on the ability of the plant to grow and thrive in a given environment that is not ideal for their growth. It is influenced by soil and climatic conditions of the soil, bioremediation does not thrive in a winter season and the disposal of the contaminants bio accumulated in the plants after harvesting is another form of pollution.[3, 8-13]

2. Crude Oil Pollution

Crude oil is described as the treasure of the world. It is a valued resource that promotes and sustains the growth of the economy of a nation, including Nigeria. Crude oil is used as energy source (fuel) to power different types of engines of ships, cars, planes, tractors and generally used in the generation of the electrical power supply of the world. In Nigeria, oil has been reported as the ‘’Life wire’’ of the Nigeria economy and is produced mainly in the Niger Delta region. Oil producing states in Nigeria include: Awka-Ibom, Bayelsa, Delta, Edo, Imo, Onto and Rivers States. The produced crude oil account for about 90% of national income revenue. Nigeria exporter of oil produce has reached 6000 barrels per day.[10]

Scientific report showed that pollution by crude oil is such an inevitable consequence of oil exploration and exploitation activities from oil producing and consuming areas caused by accidental discharge, human error, sabotage, transportation, natural causes, etc. These create several negative effects on vegetation, both directly and indirectly. Statistical scientific data showed that about 50% of spilled oil in Niger Delta area are caused by pipeline corrosion, 28% sabotage, 22% to oil production operations and engineering drills. Oil contamination in the Niger Delta has been a noteworthy wellspring of worry to the individuals living in the raw petroleum rich-territories. Oil slicks antagonistically influence the supplement level and fruitfulness status of the dirt, along these lines influencing the development of rural yields. In Nigeria, a considerable measure of unrefined petroleum is spilled every year. For instance, Research revealed around 2,000 oil spillages in Nigeria somewhere in the range of 1976 and 1988. Raw petroleum contains various natural mixes expelled as strong squanders from the petroleum treatment facility which can cause noteworthy ecological dangers.[1, 9-10]

On January 21, 2010, around 1,245 barrels of raw petroleum was spilled in Edo state following breaking down of the Nigerian National Petroleum Company gear. The spill supposedly decimated around 169,231 farmlands. Spillage of unrefined petroleum on soil makes it inadmissible for plant development because of lacking air circulation of the dirt as air is dislodged from the spaces between the dirt particles by raw petroleum. This influences the dirt and its profitability as far as the development of harvests. One of the ecological difficulties presented by oil contamination is the change of the physical and substance nature of the dirt which in this manner influences the development of plants. This adjustment is accomplished by numerous methods including the decrease of the pH substance of the dirt.[9,10,19]

The germination, development and growth of plants occur in a soil medium where air, nutrient and water resources are favourable to enhance agricultural productivity. Persistent crude oil pollution on agricultural soil and the consequent fouling effect on all forms of life, render the soil (particularly the biologically active surface layer of the soil) very unproductive and harmful to the agricultural plants. This is because; the crude oil pollution decreases the fertility of the soil in a way that their essential nutrients may not be bioavailable for the utilization by crops. The measure of crude oil toxicity on plants action is expressed in mangrove forest which over time has been dying off. Crude oil spilled on soil restricts and reduces permeability of the soil since oil is denser than water, water and air contained in the soil are been expelled by the hydrocarbon of the spilled crude oil, hence inhibiting the root of the plants from air and water absorption. The properties of soil that are involved in soil-plant-water relationship have degradable ability and include texture, infiltration, hydraulic conductivity, moisture content, pH and density, which affect root and leaf development and plant growth and yield. Crude-oil spillage has recently frequented the alluvial soils of the coastal plains of the Qua Iboe river wetlands in Akwa Ibom State, Nigeria and has deprived the residences of their socio-economic livelihood. This has fostered hostility towards the oil companies when neither the government nor the oil companies acted quickly to accommodate or alleviate effect of the degradation from the affected parts.[3-15]

The hydrocarbons in spilled crude-oil are complex and very large molecules, and they are persistent in nature and may require a strong reagent to counteract their effects on agricultural soil and to aid their degradation. When droplets of hydrophobic oil are being suspended in water, the polar molecules (water) which attract each other very strongly, in a very short time tends to squeeze out the nonpolar molecules (oil), causing them to coalesce and float to the top. When an emulsifying agent like detergent is introduced, it results into a suitable emulsion of alkyl benzene having specific gravity of about 0.856; the detergents are usually large chemical of sodium salt like sodium lauryl benzene sulfonate, with a polar head and non-polar tail, and if added to oil-water mixture in the pore and shaken vigorously, will form a fine emulsion, which is safely flushed out into drains or released into the environment.[1, 9-10]

Spilled crude oil on soil, presents unpleasant condition for the growth of plants on the soil, this is as a result of inadequate aeration as the air-filled pore-space are reduced due to the hydrophobic layer of such soils and higher demand for oxygen because of oil decomposing organisms. Pollution caused by crude oil leads to the shortage of mineral nutrients in soils and has been reported to cause such harmful effects as leaf chlorosis, necrosis, growth stunting in shoots and roots thereby leading to a shortage in accumulation of biomass. Unrefined petroleum and refined petroleum spills from tanker mishaps have harmed biological systems in Alaska, the Gulf of Mexico, the Galapagos Islands, France, the Sundarbans, Ogoniland,

and numerous different spots. The amount of oil spilled during mishaps has run from a couple of hundred tons to a few hundred thousand tons (e.g., Deep-water Horizon Oil Spill, Atlantic Empress, Amoco Cadiz), yet volume is a restricted proportion of harm or effect. Littler spills have effectively demonstrated to greatly affect biological systems, for example, the Exxon Valdez oil slick due to the remoteness of the site.[18, 21-25]

3. Common Phytoremediating Plants in Nigeria

Phytoremediation is the utilization of plants to expel contaminants from soil and water. It is a moderate yet ecologically inviting approach to evacuate poisons. Plant materials, for example, organisms, lichens, tree covering, tree rings and leaves of higher plants have been utilized for a long time to identify the statement, aggregation and circulation of substantial metals contaminations. Lower plants particularly greenery and lichens have been generally utilized because of their ability for metal collection. Some higher plants have likewise been utilized for bio monitoring of substantial metals. The following plants have the potency of phyto- remediating petroleum hydrocarbons: Sudan grass, Meyer zoysia grass, western wheat grass, big blue stem, side oats grass, sorghum, Indian grass, little blue stem, winter rye, common buffalo grass, prairie buffalo grass, bell Rhodes grass, carrot, Canada wild-rye, tall fescue, poplar tree, bush bean, switch grass, animal rye grass, perennial rye grass, round sedge, canola, oat, tilesy saga, crested wheat grass, rock sedge, water sedge, black medick, reed grass, snow willow, arctic willow, reed canary grass, alfalfa, sunflower, water leaf, pig weed white clover, red clover, wheat and tall cotton grass.[29-32]

4. Mechanistic Processes for the Phytoremediation of Petroleum Hydrocarbons

There are three basic mechanistic processes through which plants and micro-organisms remediate petroleum contaminated soil and ground water. These mechanistic processes include; degradation, containment and phyto-volatilization.

4.1. Degradation

Degradation involves the break-down of hitherto toxic substance to less toxic substances. During degradation of a petroleum hydrocarbon, species that participate both directly and indirectly are micro-organisms and plants. The following compounds are the end products of the degradation; acids, alcohol, carbon dioxide and water. In comparison to their parent compounds, these products of petroleum degradation are less harmful and they do not persist over time in a given environment than the parent compounds. Independently, both plants and microorganisms have the potency of degrading petroleum compounds. It has been suggested that it is the interaction/relationship between plants and micro-organisms (i.e., the rhizosphere effect) which is the primary mechanisms responsible for petroleum degradation in phyto-remediation processes.[3-5]

4.2. The Rhizosphere Effect

The rhizosphere is the region of soil which is closest to the roots of plants and hence, it is found directly under the influence of the root system. Plants make available root exudates of energy, carbon, nutrients, enzymes and oxygen most times to microbial populations in the rhizosphere. Root exudates of alcohol, sugars, and acids could be up to 10-20% of plant photosynthesis yearly and this provides sufficient carbon and energy to support large numbers of microbes. As a result of these exudates, microbial populations and activities are about 5 -100 times greater in the rhizosphere than in bulk soil. This plant-induced enhancement process of the microbial population is termed the rhizosphere effect and it is believed to aid in enhanced degradation of organic containment in the rhizosphere.[3-9]

4.3. Containment

Containment involves the adsorption and bioaccumulation of contaminants by plants through their roots; these contaminants tend to bind with the rhizosphere region by the help of the activities of the enzyme of the plant. This process can either be direct or indirect process. This helps in reduction of the bioavailability of the contaminants into humus or soil organic matter through a humification process and thereby increasing the soil organic matter constituents.[3]

4.4. Phytovolatilization

This involves the transfer of petroleum hydrocarbons to the atmosphere by the plants. The natural tendencies of a plant to volatilize (give-up) a contaminant that has been bio accumulated from their roots throughout their whole parts can be exploited as a natural air-stripping pump system. Phyto-volatilization is most applicable to those contaminants that are treated by conventional air -stripping i.e., contaminants with a Henry's constant KH >10 atm m^3 waterA m^{-3} air, such as BTEX, TCE, vinyl chloride and carbon tetrachloride. Chemicals with KH < 10 atm m^3 waterA m^{-3} air such as phenol and PCP are not suitable for the air-stripping mechanism because of their comparative low volatility.[16-24]

5. Phytoscreening

Phytoscreening is a fast and moderately low-cost characterization innovation that can be utilized to screen for the availability of contamination in subsurface situations. Phytoscreening depends on the examining and investigation of plant tissues (i.e., tree centre, branch, leaf) as a surrogate for testing of soil, soil gas, and groundwater. A few field studies have exhibited its value as a characterization technique, especially for unstable natural constituents. Plants interact intimately with their environment, extracting CO_2, water, macro- and micronutrients from their surroundings, even when these life essentials are available at a very little concentration. Extraction processes also collect contaminants from surrounding water, air, and soil. Innovative sampling and analytical approaches have been developed to assess the level of contamination and document pollution history. Sampling is rapid, fast, inexpensive, and causes little or no discernible damage to personal property or ecological systems we protect.[3,6,27-33]

Vascular plants develop an extensive subsurface system of roots and an expansive aerial network of leaves each having tremendous surface area. The surface area of both roots and leaves of our earth's plants have greater surface area than the footprint of earth's land surface. The extensive network linking the subsurface and atmosphere provides all nutrients, water, and CO_2 necessary for the growth of the vast majority of the earth's terrestrial biomass. Vascular plants develop an extensive subsurface root system and an expansive aerial network of leaves both with tremendous surface area. The surface area of both roots and leaves of our earth's plants have greater surface area than the footprint of earth's land surface. The extensive network linking the subsurface and atmosphere provides all nutrients, water, and CO_2 necessary for the growth of the vast majority of the earth's terrestrial biomass.[27]

Based on an understanding of plants' interactions with surroundings, plants have been used as indicators of their environments for millennia, back to Roman times when willow and poplar stands indicated the presence of a shallow groundwater table and a good location for placement of drinking wells. As plants transpire water, many dissolved constituents are Trans located concurrently. Thus, the water and wood of a tree is partly a reflection of groundwater chemistry. This concept has been applied in recent research and applications using plants as bio-indicators to map environmental pollutants termed "phyto-monitoring" or "phyto-screening'' These methods target organic contaminants in plant tissues as indicators of soil groundwater, pore water, or soil-vapor contaminants. Other research has looked at atmospheric contaminants depositing on vegetation. Plant sampling has identified atmospheric point sources and examined global distribution of persistent organic pollutants.[3,9,25]

Innovative methods have also been developed to analyse elemental concentrations in growth rings to examine local history of subsurface contamination. In many cases, contaminants are retained in the annual growth ring formed during the year of uptake. Thus, trees can preserve a contaminant uptake record over time in dated tree rings, termed "dendro-chemistry". These approaches, new sampling and analytical techniques, and the current state of knowledge are considered here and presented as a suite of methods and tools that utilize plants to delineate pollutants in the biosphere. The application of phyto-screening, phyto-monitoring and dendro-chemistry together provide innovative methods that can be used in environmental forensics investigations. When used in such a manner, the processes represent an innovative approach of phyto-forensics.[16, 23]

6. Dendrochemistry

Inorganic constituents from the atmosphere or subsurface can accumulate in plant tissue and may remain in the wood formed during the year that the contaminants were assimilated. The uptake and translocation of some inorganic constituents is under a degree of homeostatic control, particularly for essential or potentially toxic cations. In those cases, the chemistry of annual growth rings can be analysed and indicate changes in contaminant concentrations over time, termed Dendrochemistry. Target chemical accumulation in synchronous growth rings from multiple trees in the affected area provides strong evidence in assigning a date to the investigated environmental event, based on dendrochronology. The correlation between dendro-chemistry and the investigated environmental event can be further strengthened by ensuring that synchronous accumulation of a target chemical was not found in background trees outside the impacted area. The movement of some inorganic constituents across growth ring boundaries (translocation) would appear to make those constituents unsuited for dendro- chemistry and phyto-forensics. In some cases, however, mobile constituents still can provide information on the subsurface contamination if the deposition and fate within the tree are adequately understood. For example, potassium (K) is normally elevated in concentration in the outermost growth rings. In a study of groundwater contamination by organics and metals in which elevated K also was present, trees in contaminated areas Trans located excess K into the heartwood, resulting in elevated heartwood K concentrations relative to trees in uncontaminated areas. Thus, elevated heartwood K concentrations could be used as an indicator of groundwater contamination. Another example is Cl, which does not appear to move across growth-ring boundaries in situations where Cl is not at excessive environmental concentrations; however, Research found that Cl in bald cypress trees impacted heavily by salt- water intrusion was Trans located from the sapwood to the heartwood. Despite the translocation, the authors were able to estimate the timing of historical saltwater intrusion events by estimating the position of the heartwood/ sapwood boundary at the time of intrusion. The factors controlling immobilization or movement of constituents across growth-ring boundaries are not well understood currently for all target analytes. Several trees in a Maryland-based study maintained approximately the same K concentrations in the metabolically active outer growth ring, while substantial differences were present in heartwood K concentrations. The results imply that the trees may translocate some metabolically useful constituents to maintain an optimum concentration in the growth region. Studies of areas with groundwater contamination or saltwater intrusion imply that translocation sometimes can be related to depletion or excess availability of particular elements in the environment. Other as yet unknown factors are probably important also. Use of novel analytical techniques can assess the ring-to-ring concentration in the solid matrix. Use of energy dispersive X-ray fluorescence (EDXRF) and laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) have proven useful at making such analyses.[1-15]

7. Bioaugmentation

There is a growing concern about the rate of environmental degradation currently experienced throughout the world today, much of it arising from growing production and use of fossil fuels. In all continents, oil exploration and use threatens the health of the environment and living creatures including humans. An oil spill is the release of a petroleum hydrocarbon into the environment. Oil spills may be due to releases of crude oil from tankers, offshore platforms, drilling rigs and wells, as well as spills of refined petroleum products (such as gasoline, diesel) and their by-products, heavier fuels used by large ships such as bunker fuel, or the spill of any oily refuse or waste oil.[3]

Crude oil and refined fuel spills have damaged natural ecosystems in Alaska, the Gulf of Mexico, and the Galapagos Islands, France, the Niger Delta region in Nigeria and many other places worldwide. The quantity of oil spilled during accidents has ranged from a few hundred tons to several hundred thousand tons (e.g., Deep-water Horizon Oil Spill, Atlantic Empress, and Amoco Cadiz) but it is a limited barometer of damage or impact. Smaller spills have also ready proven to have a great impact on ecosystems, such as the spills experienced in the Niger Delta region in Nigeria because of the remoteness of the sites or the bottle necks hindering emergency environmental responses.[9,10,18]

The effects of oil contamination are enormous. Oil penetrates into the structure of the plumage of birds and the fur of mammals, reducing their insulating ability, and making them more vulnerable to temperature fluctuations and much less buoyant in water. Animals that rely on scent to find their babies or mothers fade away due to the strong scent of the oil. This causes babies to be rejected or abandoned, leaving the babies to starve and eventually die. Oil can impair a bird's ability to fly, preventing it from foraging or escaping from predators. As they preen, birds may ingest the oil coating their feathers, irritating the digestive tract, altering liver function, and causing kidney damage. Together with their diminished foraging capacity, this can rapidly result in dehydration and metabolic imbalance. Some birds exposed to petroleum also experience changes in their hormonal balance, including changes in their luteinizing protein. The majority of birds affected by oil spills die without human intervention. Some studies have suggested that less than one percent of oil-soaked birds survive, even after cleaning.[20-24]

For humans an oil spill can represent an immediate fire hazard. The Kuwaiti oil fires produced air pollution that caused respiratory distress. The Deep-water Horizon explosion killed eleven oil rig workers. The fire resulting from the Lac-Megantic derailment killed forty-seven and destroyed half of the town's centre, In Ikarama, Bayelsa State, Nigeria, a spill resulted in fire and subsequent burning of at least fifty personnel on the work platform.[29]

Spilled oil can also contaminate drinking water supplies. For example, in 2013 two different oil spills contaminated water supplies for three hundred thousand people in Miri, Malaysia; Eighty thousand people in Coca, Ecuador. In 2000, springs were contaminated by an oil spill in Clark County, Kentucky.[31,33]

Contamination can have an economic impact on tourism and marine resource extraction industries. For example, the Deep-water Horizon oil spill impacted beach tourism and fishing along the Gulf Coast, and the responsible parties were required to compensate economic victims.[33]

Based on available literature, oil contamination reduces the ability of soil to support the growth of plants, seeps into ground to contaminate ground water, and increases the presence of heavy metals which can bio accumulate and bio magnify causing adverse health effects. It is well known that heavy metals can be extremely toxic as they damage nerves, liver and bones, and also block functional groups of vital enzymes. Some of the metals like Nickel are also listed as possible human carcinogens (group 2B) and associated with reproductive problems and birth defects. Besides, a range of detrimental effects on fauna and flora are also well documented. Often, these contaminants also inhibit biological remediation processes due to metal sensitivity of the strain and necessitate additional combat strategies for efficient operation.

The challenge facing scientists and industrialists alike today is tackling this problem of environmental degradation in a safe, environmentally sound manner with rational cost implications. A technology that has been intensively studied is bioremediation. Although the technology has gained wide attention and studies, there is the need to investigate the trends that have evolved in studying bioremediation in the past decade; some aspects under focus are comparability of available data, the applicability of available technology, availability or unavailability of technology for laboratory investigations, geographical diversity, dearth of expertise in the field, regulatory bottlenecks associated with extensive trials and a general skepticism or acceptance of the effectiveness of the technology which may have interfered with further researches.[16-19, 24-28]

8. Bioremediation

The use of microbes in modern bioremediation is credited, in part, to George Robinson. He used microbes to consume an oil spill along the coast of Santa Barbara, California in the late 1960s. Since the 1980s bioremediation of oil spills and other hazardous wastes has received more consideration. Bioremediation is a process which uses microorganisms and their products to remove contaminants from the soil. In particular, native soil micro-organisms play a key role in soil bio-remediation as biogeochemical agents to transform complex organic compounds into simple inorganic compounds or into their constituent elements. This process is termed mineralization. The microorganisms are adsorbed to soil particles by the mechanism of ionic exchange. In general soil particles have a negative charge, and soil and bacteria can hold together by an ionic bond involving polyvalent cations.[2-7]

Bioremediation technology uses microorganisms to reduce, eliminate, contain, or transform to benign contaminants present in soils, sediments, water, and air. Bioremediation is described as the use of microorganisms to destroy or immobilize waste materials. This process of detoxification targets the harmful chemicals by mineralization, transformation, or alteration. For centuries, civilizations have used natural bioremediation in wastewater treatment, but intentional use for the reduction of hazardous wastes is a more recent development.[18-21]

Bioremediation involves the production of energy in a redox reaction within microbial cells. These reactions include respiration and other biological functions needed for cell maintenance and reproduction. A delivery system that provides one or more of the following is generally required: an energy source (electron donor), an electron acceptor, and nutrients. Different types of microbial electron acceptor classes can be involved in bioremediation, such as oxygen-, nitrate-, manganese-, iron (III)-, sulphate-, or carbon dioxide-reducing, and their corresponding redox potentials. Redox potentials provide an indication of the relative dominance of the electron acceptor classes.[3]

The presence of microorganisms with the appropriate metabolic capabilities is the most important requirement for oil spill bioremediation. The communities which are exposed to hydrocarbons become adapted, exhibiting selective enrichment and genetic changes. The adapted microbial communities can respond to the presence of hydrocarbon pollutants within hours and exhibit higher biodegradation rates than communities with no history of hydrocarbon contamination. So, the ability to isolate high numbers of certain oil degrading microorganisms from an environment is commonly taken as evidence that those microorganisms are the most active oil degraders of that environment and can be used in the bioremediation of petroleum polluted sites. Since crude oil is made of a mixture of compounds, and since individual microorganisms metabolize only a limited range of hydrocarbon substrates, biodegradation of petroleum hydrocarbon requires mixture of different bacterial groups or consortia functioning to degrade a wider range of hydrocarbons. This process depends on nutrient availability and the optimum presence of other factors that support biological functions.[19] These are:

8.1. Contaminant Concentrations

Directly influence microbial activity. When concentrations are too high, the contaminants may have toxic effects on the present bacteria. In contrast, low contaminant concentration may prevent induction of bacterial degradation enzymes.

8.2. Contaminant Bioavailability

Depends on the degree to which they sorb to solids or are sequestered by molecules in contaminated media, are diffused in macropores of soil or sediment, and other factors such as whether contaminants are present in Non-Aqueous Phase Liquid (NAPL) form. Bioavailability for microbial reactions is lower for contaminants that are more strongly sorbed to solids, enclosed in matrices of molecules in contaminated media, more widely diffused in macropores of soil and sediments, or are present in NAPL form.

8.3. Site Characteristics

Have a significant impact on the effectiveness of any bioremediation strategy. Site environmental conditions important to consider for bioremediation applications include pH) with an optimum in the range of 6-8, temperature, water content, nutrient availability, and redox potential.

8.4. Redox Potential and Oxygen Content

Typify oxidizing or reducing conditions. Redox potential is influenced by the presence of electron acceptors such as nitrate, manganese oxides, iron oxides and sulphate.

8.5. Nutrients

Are needed for microbial cell growth and division. Suitable amounts of trace nutrients for microbial growth are usually present, but nutrients can be added in a useable form or via an organic substrate amendment, which also serves as an electron donor, to stimulate bioremediation.

8.6. Moisture Content

Microbial growth requires an optimum presence of water in the environmental matrix. For optimum growth and proliferation, microorganisms require 12% to 25% of moisture.

8.7. Temperature

Directly affects the rate of microbial metabolism and consequently microbial activity in the environment. The biodegradation rate, to an extent rises with increasing temperature and slows with decreasing temperature.

9. Kinds of Bioremediation

Feasibility of bioremediation depends on the location of contaminants. Approaches for implementation of bioremediation depend on whether the impacted soil to be treated is intact in the environment or it is to be excavated for treatment in an offsite facility. If on site, the term *In situ* remediation suffices and if offsite, it is described as *Ex situ*. Some authors have used this to describe the type of bioremediation. However, it is necessary to determine what exactly is done *in situ* and *ex situ* and use same to describe the types of bioremediation.

10. Biostimulation

Hydrocarbon biodegradation in soil can be limited by many factors, including nutrients, pH, temperature, moisture, oxygen, soil properties and contaminant presence. Bio stimulation involves the modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (e.g. in the form of molasses), which are otherwise available in quantities low enough to constrain microbial activity. It was described as the addition of nutrients, oxygen or other electron donors and acceptors to the coordinated site in order to increase the population or activity of naturally occurring microorganisms available for bioremediation.[5]

Bio-stimulation is a type of natural remediation that can improve pollutant degradation by optimizing conditions such as aeration, addition of nutrients, pH and temperature control. They opined that bio-stimulation can be considered as an appropriate remediation technique for petroleum pollutants removal in soil and requires the evaluation of both the intrinsic degradation capacities of the autochthonous micro flora and the environmental parameters involved in the kinetics of the in situ process.[1-7]

The primary advantage of bio-stimulation is that bioremediation will be undertaken by already present native microorganisms that are well-suited to the subsurface environment, and are well distributed spatially within the subsurface. The primary challenge is that the delivery of additives in a manner that allows the additives to be readily available to subsurface microorganisms is based on the local geology of the subsurface. Tight, impermeable subsurface lithology (tight clays or other fine-grained material) make it difficult to spread additives throughout the affected area. Fractures in the subsurface create preferential pathways in the subsurface which additives preferentially follow, preventing even distribution of additives. Addition of nutrients might also promote the growth of heterotrophic microorganisms which are not innate degraders of Total Petroleum Hydrocarbon thereby creating a competition between the resident micro flora.[19-28]

11. Bioaugmentation

Since the 1970s, Bioaugmentation, or the addition of oil-degrading microorganisms to supplement the indigenous populations, has been proposed as an alternate strategy for the bioremediation of oil contaminated environments. The rationale for this approach is that indigenous microbial populations may not be capable of degrading the wide range of potential substrates present in complex mixtures such as petroleum or that they may be in a stressed state as a result of the recent exposure to the spill. Other conditions under which bio-augmentation may be considered are when the indigenous hydrocarbon-degrading population is low, the speed of decontamination is the primary factor, and when seeding may reduce the lag period to start the bioremediation process. For this approach to be successful in the field, the seed microorganisms must be able to degrade most petroleum components, maintain genetic stability and viability during storage, survive in foreign and hostile environments, effectively compete with indigenous microorganisms, and move through the pores of the sediment to the contaminants.[18]

Different microbial species have different enzymatic abilities and preferences for the degradation of oil compounds. Some microorganisms degrade linear, branched, or cyclic alkanes. Others prefer mono- or polynuclear aromatics, and others jointly degrade both alkanes and aromatics. The study of microbes in bioremediation systems makes possible the selection of microorganisms with potential for the degradation and production of compounds with biotechnological applications in the oil and petrochemical industry.[27-29]

Successful bio-augmentation treatments depend on the use of inocula consisting of microbial strains or microbial consortia that have been well adapted to the site to be decontaminated. Foreign microorganisms (those in inocula) have been applied successfully but their efficiency depends on ability to compete with indigenous microorganisms, predators and various abiotic factors. Factors affecting proliferation of microorganisms used for bio-augmentation including the chemical structure and concentration of pollutants, the availability of the contaminant to the microorganisms, the size and nature of the microbial population and the physical environment should be taken into consideration when screening for microorganisms to be applied.[9]

Bio-augmentation involves the introduction of microorganisms isolated from the contaminated site, from a historical site or carefully selected and genetically modified to support the remediation of petroleum hydrocarbon contaminated sites based on the assumption and/or confirmation that indigenous organisms within the impacted site cannot biodegrade petroleum hydrocarbon.[17]

12. Recent Bioremediation Method

The use of certain genetically engineered microorganisms to influence their ability to utilize specific contaminants such as hydrocarbons and pesticides is gaining grounds. This technique had an early mention in the late 1980s and early 1990s. The ability to 'engineer' microorganisms to improve degradative properties is based a possibility to explore genetic diversity and metabolic versatility of microorganisms.[8-12]

The blueprint necessary for gene encoding for biodegradative enzymes is present in chromosomal and extra chromosomal DNA of such microbes. Recombinant DNA techniques explore the ability of an organism to metabolize a xenobiotic by detecting the presence of degradative genes and transforming them into appropriate hosts through a suitable vector within a controlled setting. This technology explores Polymerase Chain Reaction (PCR), anti- sense RNA technique, site directed mutagenesis, electroporation and particle bombardment techniques.[21]

The first step in Genetically Modified Microorganism (GMM) construction is selection of suitable gene(s), next, the DNA fragment to be cloned is inserted into a vector and introduced into host cells. The modified bacteria are called recombinant cells. The next step is production of multiple gene copies and selection of cells containing recombinant DNA. The final step includes screening for clones with desired DNA inserts and biological properties.[21]

Since the possibility of conferring new properties into existing organisms abound, researchers have studied the ability of modified organisms to degrade petroleum hydrocarbons or hydrocarbon based compounds. For example, Recent study showed transfer of plasmid pJP4 from two introduced donors, natural host *R. eutropha JMP134* and laboratory-constructed *strain Escherichia coli D11*, to indigenous microbes in soil contaminated with 2,4-D. A key difference between donors was their ability (*R. eutropha*) or inability (*E. coli*) to mineralize 2,4-D. Additionally, they studied transconjugant occurrence, their identification and plasmid persistence. Both inoculated donors were detectable and they transferred plasmid pJP4 to indigenous recipients to different extents. In the first experiment 2, 4-D was degraded significantly faster (28 days) in soil inoculated with *R. eutropha JMP134* as compared with soil inoculated with *E. coli D11* (49 days). Interestingly, a greater number of trans conjugants was detected in *E. coli D11*-inoculated soil and they were members of the *Burkholderia* and *Ralstonia* genera. After reamendment 2, 4-D was degraded more rapidly in the soil with *E. coli D11*inoculants than in *R. eutropha JMP134*-inoculated treatments. These results indicated that choice of donor microorganism is a crucial factor to be considered for bioaugmentation approach.[20-25,33]

In an initial study, The University of Tennessee in collaboration with Oak Ridge National Laboratory performed field based bioremediation using Genetically Modified Microorganisms. The organism involved was *Pseudomonas fluorescens* strain designated HK44, released into a hydrocarbon contaminated environment. The original parental strain from with the strain HK44 was derived was isolated from a manufactured gas plant heavily contaminated with polyaromatic hydrocarbons (PAHs). The naphtalene catabolic plasmid (PUTK21) was introduced into the strain to form *P. fluorescens HK44*.

Upon introduction of the modified organism to naphtalene (or the intermediate metabolite salicylate) there was an increased catabolic gene expression, napthalene degradation and a coincident bioluminescent response. Because of well-established tools from metabolic engineering and biochemistry it is possible to infuse different pathways into a 'designer' microbe. This technique is a very powerful approach to enhance petroleum hydrocarbon biodegradation. Very often, these pathways are combined with existing pathways to enable complete biodegradation. The construction of a hybrid strain which is capable of mineralizing components of a mixture of benzene, toluene and p-xylene simultaneously was attempted by redesigning the metabolic pathway of *Pseudomonas putida*. A hybrid strain expressing both the tod and the toll pathways was constructed and was found to mineralize a benzene, toluene and p-xylene mixture without accumulation of any metabolic intermediate.[32-33]

Irrespective of the glowing prospects of genetic engineering to confer new properties on microbes and subsequently improve their abilities on the field, the practice is faced with some constraints. Report showed that there is difficulty in deducing the exact extent the microbe under modification actually contributes to the degradation process, recognizing that factors such as volatilization and chemical transformation simultaneously occur within a reactor system. In using GMM, it can be problematic distinguishing between GMM specific degradation and biodegradation due to the presence of indigenous microbial consortia. Another obstacle is an inability to statistically conclude on bioremediation efficiency because of the highly heterogeneous distribution of the contaminants. Sample-sample chemical analyses can typically vary by up to 200% making valid conclusions blurred. To buttress this, in an experiment using P. *fluorescens HK44* lysimeter release, soil PAH concentrations were dispersed heterogeneously ranging from 0.04 to 192ppm spatially. Consequently, a precise evaluation of the effectiveness of P. *flurescens HK44* in the overall process could not be adequately determined. Statistical models that can incorporate chemical heterogeneity kinetics into the entire design are required before valid efficacy assessments can be obtained.[33]

Another impediment to actualizing field release studies is the securing of the required governmental permission, which is often a difficult and lengthy endeavour. Although necessary to ensure environmental and public health safety, the process often leads to an overall aversion to GEM implementation in environmental systems, with researchers concentrating rather on the optimization and commercial development of naturally occurring (intrinsic)microbial degradation. Also, during the approval process the GEM might undergo significant refinement and genetic restructuring while in the hands of researchers, making the originally proposed release microorganism somewhat obsolete. This unfortunately prevents the integration of state-of-the-art engineered microbes into field release studies.[33]

13. Conclusions

Environmental contamination causes several human effects which could be fatal and it has been a major source of human health risk throughout the world. Heavy metals and crude oil contamination are some of the major environmental pollutions which are of public concern. Their health effects have been in existence over time, exposure to crude oil contamination and heavy metals have been on rise at different parts of the world. Phytoremediation is cost effective and aesthetically pleasing because the plants can be easily monitored and metals absorbed by the plants may be extracted from harvested plant biomass and then recycled. It helps to conserve the environment, it combats the spreading of contaminants in air and soil, it is suited to remediate a very large area of soil, and the process is amenable to a variety of inorganic and organic compounds. However, a major con of phytoremediation is that it is limited to the surface area and depth occupied by the roots. It requires long-term commitment, because the process depends on the ability of the plant to grow and thrive in a given environment that is not ideal for their growth.

Conflicts of Interest

The authors declare no conflict of interest.

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