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REVIEW ON CRUDE OIL/PETROLEUM SPILL AND ITS BIOREMEDIATION STRATEGY

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Abstract: Oil spillage has become a serious environmental problem with increasing resource exploitation, transportation, storage, and accidental leakage of oil on lands and water. Several approaches such as biological, chemical and physical/mechanical methods are utilized to restore petroleum-polluted lands and water to their natural status. The usual traditional methods such as chemical and physical processes sometimes do more harm to the terrestrial and aquatic ecosystems. Hence, the need for the development of Bioremediation (biological treatment technique), an eco-friendly process for oil spill remediation.

Bioremediation is a process or strategy that uses biological agents, principally, microorganisms in detoxifying or cleaning up contaminated environments. It is a promising option as it is effective and economic in removing petroleum spills with minimal environmental challenges. However, it is a relatively slow method and the degree of success depends on the properties and fate of oil spilled in the environments, selected microorganisms, environmental limitations, and extraneous surrounding complications of the process. The microbial factors include bioavailability of hydrocarbons, mass transfer through the cell membrane, and metabolic limitations. In this review article, I was able to lay bare the prospects of using bioremediation in the clean-up of crude oil against the background of constraints that could interfere with the effectiveness of the process. Furthermore, this article has presented a concise account of other

conventional remediation strategies/approaches, weighed their advantages and disadvantages in the context of remediation of crude oil spill.

Keywords: Crude Oil, Remediation, Bioremediation, Biostimulation, Bioaugumentation Contamination, Microorganisms, Daltons, Avogadro

Introduction

Land in its natural ecological structure is a basic and irreplaceable natural resource which provides various ecosystem services and is the paramount connection between the components-air, bedrock, water and biota that constitute our environment. These components interact with one another to constantly provide food, fuel and fiber to support organisms.[1] However, historic and ongoing anthropogenic activities have led to large-scale contamination of lands, and these have resulted in useful lands being transformed to wastelands.[2] Owing to the problem of environmental petroleum contamination resulting from oil exploration, transportation and processing, there is a need for remediation and restoration of the operational status of all ecosystems, most especially land (soil) or water bodies. Soil, being the indispensable depositing domain for all pollutants entering all components of ecosystems, the issue of remediation of petroleum-contaminated soil is one of the main environmental challenges facing man and his environment presently. It is one of the most diverse environmentally damaging pollution problems posing potential threats to man and localized ecosystems.[3,4] In previous times, chemical and physical strategies have been used for remediation of petroleum contaminated lands such as soil washing, solidification, thermal treatment and stabilization. However, these techniques are financially burdensome, disruptive to the environment and also involve intensive energy consumption.[5] Hence, natural/biological remediation methods such as bioremediation and phytoremediation have been developed to provide more eco- friendly and cost-effective clean-up of sites marred by petroleum spills.[6,7]

Petroleum Hydrocarbons

Petroleum is defined as a broad range of hydrocarbon-based compounds comprising of specific distillates of crude oil, and organic oil such as animal fats vegetable oils, and synthetic compounds. Crude oil, natural gas, tars and asphalts are examples of petroleum hydrocarbons.[8] The word "petroleum" signifies "rock oil" or "oil from the earth".[9] Petroleum, which is widely used in our daily lives, plays a crucial role in our modern world economy. We depend on it in many ways because it provides great benefits to our society. It is not only the main source of energy for manufacturing, transportation and heating, but also acts as a raw material for many individual components in common household and industry products used on a daily basis. [127]

So it is vitally important to view known remediation methods as options to correcting the incident of spills through accidental or natural events, simply because there are few replacement options at this time in other material compositions that are available to us at the scale petroleum distillates can provide. [127]

Petroleum hydrocarbons (PHCs) are utilized to describe and explain mixtures of organic compounds located in or derived from geological substances such as oil, coal and bitumen.[10] They are generally divided into two groups- Aliphatics and Aromatics. Aliphatics include alkanes that have single bonds between carbon atoms having the homologous series formula of C_nH_{2n+2}. Alkenes, which contain one or more double bonds between atoms, have formula of C_nH_{2n}, and cycloalkanes which contain carbon atoms in their cyclic structures.

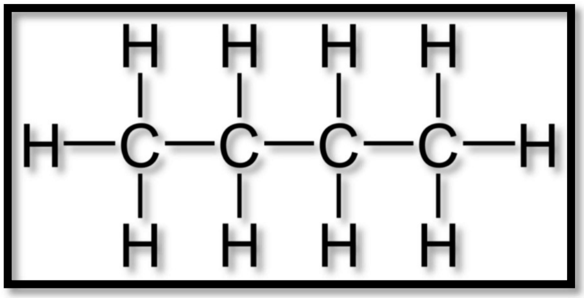

Figure 1 Simple Aliphatic compound [128]

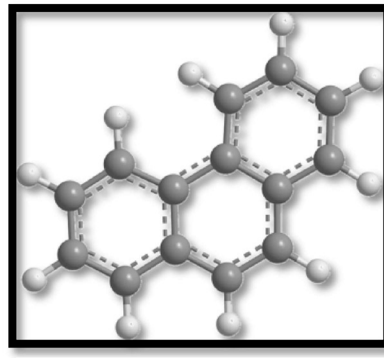

Figure 2 An Aromatic Compound showing the delocalized electron cloud in a dotted line[128]

They are comprised of two categories: gasoline range organics which refers to small chain alkanes (C6-C10) [ethane, methane, propane], volatile aromatic compounds (BTEX) and common oxygenates (MTBE and ethanol); and diesel range organics comprising of the alkanes with longer chains (C10-C40) or non-halogenated semi-volatile organic compounds and hydrophobic chemicals such as PAHs (phenanthrene, naphthalene, anthracene, benzo[a]pyrene).[8,11] The small chain alkanes normally have low boiling points between the range of 60 and 170 ^{0}C.[11]

Aromatics possess one or more benzene rings in their structures. Monoaromatics are aromatics having one benzene ring as part of their structures, while the polycyclic aromatic hydrocarbons (PAHs) are aromatics possess two or more fused benzene rings. Some of the most common aromatic compounds in petroleum are monoaromatics, such as benzene, toluene, ethylbenzene, and xylenes (BTEX).[4,12-14] Gasoline contains more BTEX than Crude oil, but on average, BTEX compounds represents about 2% by weight of crude oil when the average weights for separate BTEX compounds are combined. Normally, crude oil contains higher concentrations of aliphatic hydrocarbons and lower concentrations of aromatic hydrocarbons.[15] Usually, aromatic and saturated (Paraffin) hydrocarbons account for 75% of the mass of crude oils.[15,16,17] Generally, aromatic compounds are more toxic than the aliphatic compounds and their toxicity has been found to vary with size.[17,18,19]

Natural gas condensates constitute primarily of aliphatic hydrocarbons. However, the condensates may contain a reasonable amount of BTEX compounds. Basically all aromatic compounds have a sweet, pleasant odour, but most aliphatic compounds are odourless.[127]

Impact of Petroleum/Crude Oil Contamination on Ecosystems

It has been stated that petroleum spills can occur in marine and terrestrial environments and can pose threat to the ecosystem and human health.[20] They also have a negative environmental impact on ecosystems by harming or killing wildlife and plants, contaminating habitats and food chain, with their influence on ecosystems being direct or indirectly. Direct forms according to the U.S. Environmental Protection Agency report, "petroleum spills threaten public health and safety by polluting drinking water, causing fire accidents and explosive hazards, diminishing air and water quality, negatively affecting agricultural activities, destroying recreational areas and wasting nonrenewable resources.[21] Suspected indirect forms include long term health related poisoning through carcinogens and with Volatile Organic Compounds (VOC) inadvertent influence to air, water, and transfer through wildlife and other food supplies.

The extent of harm that petroleum can cause to human life and ecosystems varies with the quantity and concentrations of it spilled, site factors (i.e. vegetation, terrain, climate and soil texture), management and released time.[22] Although the contamination is usually caused by a mixture of hydrocarbons, aromatic compounds tend to be more harmful and poisonous than the aliphatic compounds.[23] In aquatic toxicology, it is now well-established that the chemical characterization of pollutants exposure is not sufficient and that multidisciplinary approaches coupling chemistry and biology have to be developed to allow linking of the presence of contaminants and their putative toxic impacts.[129] The costs of these environmental damages such as Deepwater Horizon and Exxon Valdez are immeasurable and can adversely influence wildlife and human health. The mutagenic and carcinogenic effects caused by petroleum pollution in water bodies have been demonstrated. Whenever marine petroleum spills occur, it prevents oxygen penetration and light diffusion in the bottom layers of seas.[24] In addition, oily layer on water surface threatens the existing and future marine flora and fauna.[25] The adverse effects of petroleum spills on the sea and fresh water include contamination of sea food, destruction of wildlife, and reduction of tourism industry.[26]

The physical attributes of petroleum oil and grades each contain molecular weights that can have adverse effects on microscopic food supplies for ocean plankton, both Phytoplankton (plants) as well as Zooplankton (animals). The lighter the petroleum on the surface of the water the easier it is to separate Aromatic hydrocarbons that can leach from oil while covering and churning with the ocean surface causing phytoplankton to absorb these chemicals which are in turn eaten by zooplankton followed by larger species. More research needs to be done to determine the severity of plankton acting as hosts to transport nano-particles of toxins. The problem is really three-fold: the phytoplankton are starved from sunlight which is unable to pass through the oily sometimes reflective film, followed by zooplankton consuming half-dead or rotting phytoplankton that can be tainted with leached chemicals. The third, more bizarre congruent element is the fact that phytoplankton live near the surface in order to benefit from sunlight, whereas zooplankton live deeper during the day to avoid predators, and then rise to feed on the phytoplankton at night. The darker and more dense the layer of petroleum on the surface of the water, then fooling the zooplankton to rise during the day and becoming easy prey for species that feed on them. The word "plankton" comes from the Greek word planktos for "drifter" or "wanderer", basically organisms that do not have the power to swim against currents or tides but flow with it which can allow them to float under a spill. Phytoplankton consumes carbon and nitrogen and converts it into oxygen through the use of sunlight. Plankton is incredibly important to the ocean ecosystem, and very sensitive to changes in their environment, including in the temperature, salinity, pH level, & nutrient concentration of the water and they produce up to an estimated 90% of the oxygen on Earth. [130]

This leads us to believe disruption to the food chain in both localized fresh water and oceanographic settings from water borne oil spills can be:

- detrimental to Zoo & Phytoplankton from toxin absorption
- depletion of oxygen through a symptom similar to eutrophication
- as well as high depletion of zooplankton falling easy prey to predators caused by false signals from inadvertent darkening of the water, emulating nightfall allowing the zooplankton to react to the perceived night signal.

Of course as the spill is localized, this scenario would be self healing once the initial spill threat has dissipated over extended periods of time by natural means or is removed through intervention, however the speed at which the oil spill is removed would be the determining factor as to the extent of damage to the natural cycle of life of Zoo & Phyto genre.

The toxicology aspect of this event is that marine phytoplankton are a "uni-cellular" organism — like bacteria, it exists in extremely small nano-sized particles. It is confirmed that phytoplankton can become a host carrier that may not harm them individually and can carry the toxin to larger fish and mollusks, eventually humans, such particular heavy metals called mercury and lead which occurs naturally in nature, but are highly present in crude and petroleum oil. [138] Animals including humans that consume fish products only require small nano-sized particles to be readily absorbed into the bloodstream, so collected particles of metals like mercury continue to form into methyl-mercury which can be highly toxic to human brain function. [131] The greatest concern is filter feeders like Bivalve Molluscs or mussels that feed on Phytoplanton and Zooplankton that may be tainted with these toxins. Enterobacter sp. EMB21 seems promising for heavy metal remediation wherein the remediated metal can be trapped inside the cells. The process can further be developed for the synthesis of valuable high-end functional alloy, nanoparticles, or metal conjugates from the metal being remediated. [138]

According to the Centers for Disease Control and Prevention, there is no safe level of lead exposure for children. Exposure is linked to devastating long-term harm in children, including brain and nerve damage, stunted growth and development, learning and behavior problems, hearing and speech problems, lower IQ and poor school performance.[132] Microbes get energy from the contaminants by breaking chemical bonds and transferring electrons from the contaminants to an electron acceptor, such as oxygen. [137]

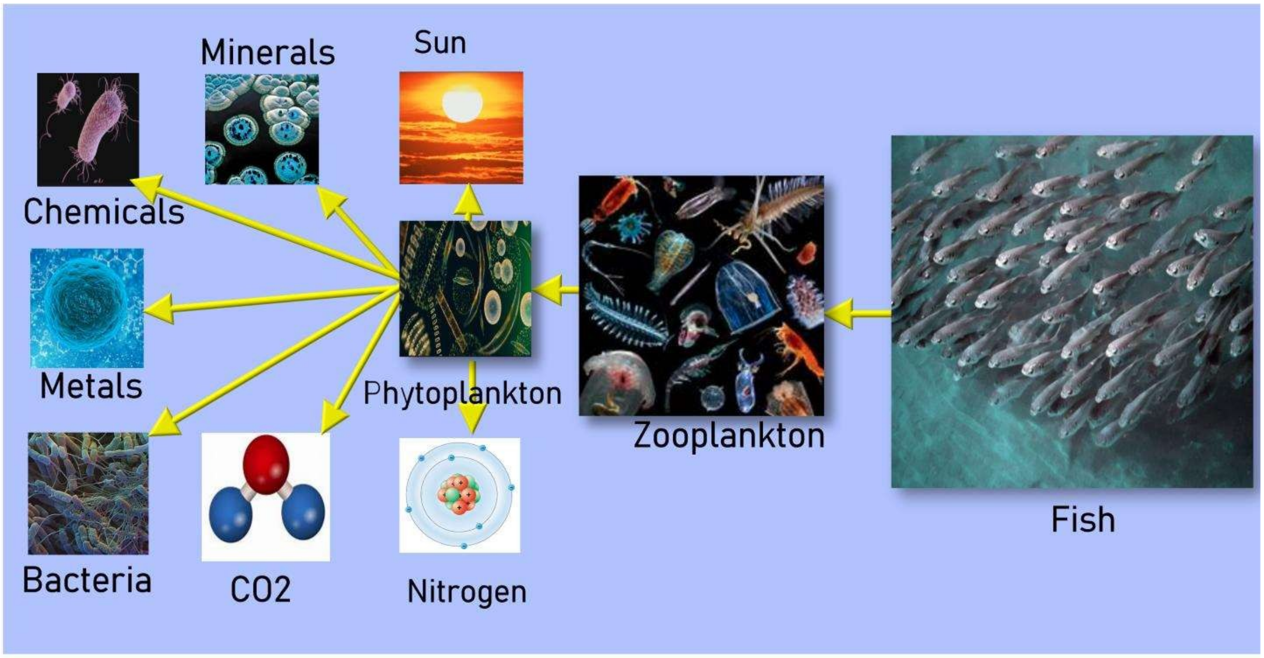

Figure 3 Zoological transports of heavy metals from oil deposits into food supplies

Petroleum Hydrocarbon pollution of land is a concern for a number of specific reasons as figure 5 depicts. First, when Petroleum hydrocarbon (PHC) is released into the soil, its volatility can pose a fire or even explosive hazard as mentioned earlier, mainly when vapors enter confined spaces. Second, pollutants can interfere with the nutrient cycle and water transmission, thus leading to land degradation. Third, degraded petroleum residuals may bind to soil particles and be retained for years. Although these contaminants may be of immense benefit to oil degraders as a carbon source, they are yet toxic to the majority of soil organisms. Some researchers are of the view that, PHC contaminants are capable of altering the ecology and the physiology of bacteria and fungi.[27] More so, PHCs may destroy the aesthetic nature of an environmental media by inducing repulsive odour, taste or appearance to it. Lastly, just as Petroleum hydrocarbon pollution of soil is a concern; it is also a potential threat to other ecosystems. If the PHC release is dominant at a place, it continues to extend its adverse effects to adjacent areas as individual compounds continue to disentangle and migrate away from the contaminated area via air or groundwater.[28] As a result, PHCs and other chemicals can find their way to drinking water and so pose a severe threat to human health and the ecosystem.

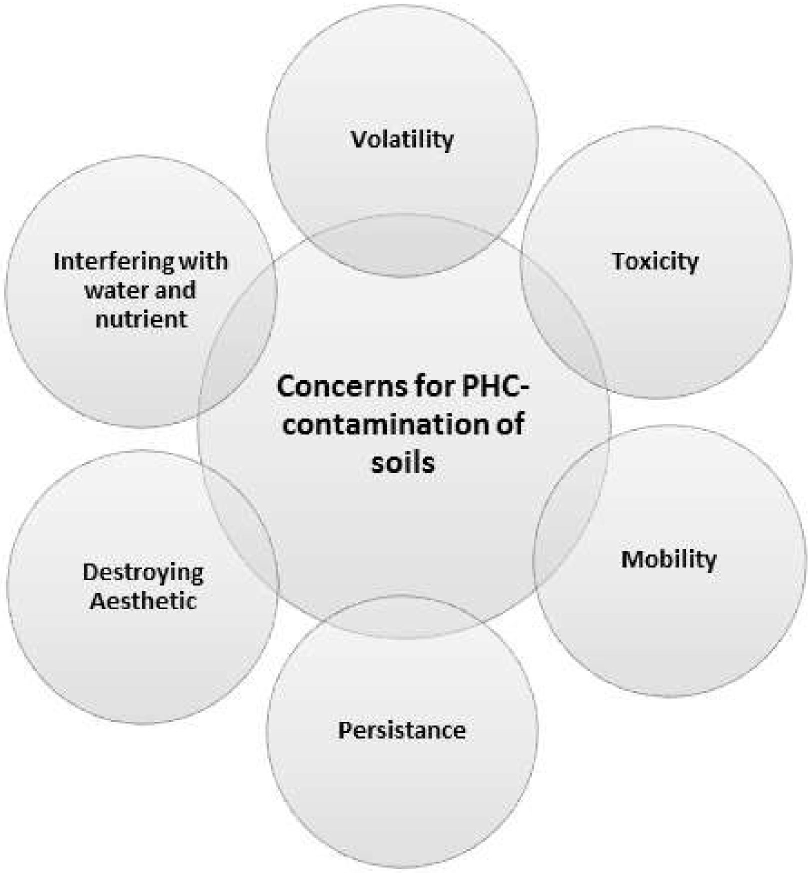

Figure 4 Showing concerns over Petroleum Hydrocarbon-Pollution of soils as modified by the Canadian standards for petroleum hydrocarbons (PHC) in soil.[10]

Sources of Petroleum Contamination

With the advent of the petroleum industry, petroleum pollution of soil via crude oil exploration, transportation, processing and consumption is highly unavoidable. Most of the Petroleum Hydrocarbons (PHCs) obtained from crude oil or refined oil products released to the soil environment are mostly through human activities, as industrial by-products or as by-products from domestic or commercial uses.

In different countries of the world, the principal sources and contaminating activities may vary from one another. However, treatment and disposal of waste as well as industrial and commercial activities have been reported to be the most main sources of oil pollution in Europe.[29] In Nordic countries where the climatic conditions favour a few coniferous and broadleaf tree genera, petroleum hydrocarbons empty into the boreal ecosystem via surface spills or leakages from storage tanks and pipelines.[30,31]

Petrochemical Hydrocarbon Genre

Each spill has the potential for varying degrees of toxicity contaminating localized environments that is found in the processes of refining. Handling and transportation of these various grades prove challenging as, it is assumed lighter grades have extremely high rates of dispersity in a short period of time, whereas the heavier the PHC then the more localized the contamination but can be higher rates of remediation per square meter.

The mass of the substance in the spill, measured in Daltons or Unified Atomic Mass Units could and should play an important role in determining how best to approach cleanup methods, whether bio, phyto, chemical, manual, or other methods of cleanup. Visual interpretations of the cleanup do not determine the micro aspects of a spill measured in mole.[132]

Figure 5 Courtesy of "Oilsands Magazine"

Lighter refined grades can spread to a much larger footprint in a very short period of time creating a thinner sheen on water surfaces, and consequently can be absorbed quickly into soils. This is dependent on temperature and weather at the time of the spill. On water, inclement weather can cause the spill to break apart causing pockets of PHC making it more difficult to control the entirety of the spill, whereas on land with the use of large volumes of moisture the spill can migrate to vast areas of land, eventually making its way into runoff channels and fresh waterways. The more moisture encountered at the time of the spill, the quicker the flow and the less control you have of the situation.

As well the degree of harmful and toxic residues in the thicker grades of the PHC can have dire effect on flora and fauna as do grades that are more refined and have higher polymerization methods. Oil that is transported is broken down into 5 grades ranging from:[134]

1. non-persistent light oils
2. persistent light oils
3. medium oils
4. heavy oils
5. sinking oils

Each of these oils has different evaporation rates and levels of severity to eco systems, but the importance of this is where bioremediation fits in best during or after other forms of mechanical measures are taken. Bioremediation can be an important component to remediating soil, water, as well as water body basins. [134] The concern in all of this is how the dissolution of toxins and isotopes in the PHC combined with the sometimes violent churning of larger bodies of water due to wind and weather, the greater and more persistent the churning effects, then the greater the potential in Avogadro Constant.

In the early 1900's Einstein working on the principles of Avogadro Constant reworked the calculations and developed a formula that linked the change in viscosity to the total volume of molecules dissolved per unit volume of solution. This formula could be re-organized to give

$$a^3 N = \frac{3M}{10\pi\rho}\left(\frac{\eta*}{\eta} - 1\right),$$

Figure 6 Einstein's formula of Avogadro's Constant [135] where M is the molecular weight of the dissolved molecules, a is the radius of the particles, ρ is the mass of the dissolved substance per unit volume of solution, η is the viscosity of the solvent and η^* is the viscosity of the solution. [135]

In the case of oil spill on land complications arise due to the absorption rate of soils through hard pack or loose top soil, as well as the moisture content within the soil. An experimental model pouring oil on wet and dry soil revealed results that immediate treatment must be performed in both systems but particularly in the wet system, due to its higher initial penetration rate. [136] Immediate response to oil spills is most effective as shown above with land based spills but there remains the lingering problem of how to effectively remediate oil contaminated soil even if the soil is removed from the site. Bioremediation can be a very effective solution to repurposing extrapolated overburden, regenerating areas difficult to contain or repurpose, as well as general biological maintenance of questionable parcels. Although existing microorganisms can detoxify a vast array of contaminants, some compounds are more easily degraded than others. In general, the compounds most easily degraded in the subsurface are petroleum hydrocarbons. [137]

Historical Cases of Crude Oil Spill Accidents

An oil spill is the leakage of crude oil onto land surfaces and water bodies. Several global oil spill accidents have remained sources of environmental and health hazards years after, owing to the fact that some of the spilled oil was not completely cleaned-up. They are caused mainly as a result of intensified petroleum exploration and production and poor handling. These oil spills have altered man's ecosystem within the affected sites and rendered them unacceptable for development and aesthetics, not to mention unexplored health concerns for both human and wildlife aspects. Some of these historical oil spills have been presented as follows:

Torrey Canyon Oil Spill

It was a devastating oil spill incident that occurred in 1967 in the United Kingdom. This happened when Supertanker SS Torrey Canyon spilled an estimated 25-36 million gallons (94-164 million liters) of crude oil.[32] Attempts to reduce the damage included the bombing of the wreck by aircraft from Royal Navy and Royal Air Force, causing a potential international incident, as the ship was not British, and was in international waters.[33] Hundreds of miles of coastline in Britain, France, Guernsey and Spain were affected by the oil and other substances used in an effort lessen the damage.[34] At the time, it was the world's worst oil spill, and led to significant changes in maritime law and oil spill responses. It remains the worst spill in UK history.

Amoco Cadiz Oil Spill

This oil spill happened on the morning of 16th March, 1978, when a very large crude carrier (VLCC) stocked with nearly 69 million gallons of light crude oil, ran aground on shallow rocks off the coast of Brittany, France.[33] The disastrous incident contaminated large stretches of the Brittany shoreline in France, releasing 227,000 tons of light crude oil and bunkers in marine and also contaminated 320 km of the shoreline length up to a depth of 20 inches.[35] About 200 miles

(321 km) of French coast was polluted by the oil slick, which killed millions of invertebrates, such as mollusks and crustaceans, estimated 20,000 birds, and contaminated oyster beds in the region.[36]

Exxon Valdez Oil Spill

Figure 7 Exxon Valdez Oil Spill Trustee Council/NOAA (ID: line1532) [Picture taken from John P. Raffert, Encyclopedia Britannica Editor]

The Exxon oil spill took place in March, 1989 when a tanker crashed a reef in Alaska, USA which emptied thousands of tons of oil into the sea.[25] The sea and shoreline contamination caused severe localized ecological damage to nearby community.[37] It was reported that more than 250 thousand seabirds were killed due to this spill.[38] Figure 2 is an image of Exxon Valdez oil spill, where bioremediation was applied effectively as a strategy.

Mega Borg Oil Spill

This spill happened in June 1990, in the Gulf of Mexico roughly 50 miles off the coast of Texas, when an oil tanker, Mega Borg caught on fire and exploded. Texas General Land Office reported that seed culture produced by Alpha Corporation proved effective in cleaning up a reasonable quantity of spilled oil, though, no independent monitoring was applied. Bioremediation was the first practical clean up strategy applied on the affected region.[33]

Apex Barges Oil Spill

This is a major oil spill accident involving 692,000 gallons of catalytic feedstock oil that took place at Galveston Bay in Texas in July 1990, when a Greek tanker ship Shinoussa collided with and sank the tank barge Apex 3417 that damaged the tank barge Apex 3503 in the Houston Ship Channel.[39] Application of bioremediation to this spill using seed culture from Alpha Corporation was reported to be successful by Texas General Land Office. However, an independent observation revealed that although the treated oil changed in physical appearance, chemical analysis could not prove that the product enhanced petroleum biodegradation.[37]

Gulf War Oil Spill

The Gulf War oil spill was one of the largest oil spills in history, resulting from the Gulf War in 1991. The report from the incident revealed that in an effort to prevent U.S. forces from landing on the beaches of Kuwait, Iraqi forces intentionally dumped oil into the Persian Gulf. They released oil from eight oil tankers, a refinery, two terminals, and a tank field.[40] Having anticipated an amphibious invasion, Iraqi forces also dug long trenches down the coastline and filled them with oil.[41] The entire act of environmental terrorism released a total of 11 million barrels of crude oil into the Gulf, resulting in the largest oil spill in history.[40] Oil continued spilling into the Gulf at a rate of up to 6,000 barrels a day for three months. In addition, while the Iraqis were retreating, they set ablaze a reported 732 oil wells.[41] When the Kuwait Oil Company first announced this in May of 1991, they calculated that the oil wells were burning at the rate of 6 million barrels a day. This is as shown in Figure 3.

Figure 8 Arabian Gulf War Oil Spill (image from Wikimedia commons)

Mingbulak (or Fergana Valley) Oil Spill

This was the largest inland oil spill, and Asia's worst oil spill, which took place on 2 March, 1992 in Fergana Valley Uzbekistan. A blowout from an oil well near the valley expelled oil into the valley releasing 88 million gallons of oil into land.[36] The oil caught fire and burned for two months before the well pressure subsided.[42] The ground absorbed the spill, and no clean up was feasible.[43]

Atlantic Empress Oil Spill

The oil spill was the fifth largest on record and the largest ship-based spill that occurred on July 19, 1979 in Trinidad and Tobago. It happened as a result of the collision between the VLCCs Atlantic Empress and the oil tanker, Aegean Captain during a tropical storm, releasing 287,000 metric tonnes of crude oil into the Caribbean Sea.[44] Both ships caught fire, and the fire ignited an oil slick, however, the Atlantic Empress caught the worst of the collision. The Atlantic Empress was towed away from land, and it burned for two weeks before it sank. In contrast, the fire aboard the Aegean Captain was extinguished, and the vessel was towed to Trinidad. In spite of the enormous volume of oil released during the accident, the spill caused very little environmental damage to the beaches on nearby islands; winds pushed most of the oil out to sea, where it dispersed. However, 27 sailors died during the accident.[45] The remaining crew from both ships was taken to Tobago for medical treatment, while the Empress's captain was transported to a hospital in Texas, having inhaled fire.[46]

The Kolva River Spill

This Kolva River, which was one of the ten largest oil spill occurred in1994 in the city of Usinsk, Komi Republic, Russian Federation. A badly corroded pipeline within Kharyaga oil field and the town of Usinsk had been leaking for eight months, but was contained by a ditch erected around the leak site. However, the collapse of the dam caused by severe weather conditions resulted in the spillage of 2 million barrels of oil across the fragile Siberian tundra and into the Kolva River, a tributary of the Pechora River which flows into the Barents Sea.[47] Twenty-three species of fora and fauna were affected by the disaster which polluted 186 km^2 of grassland and marshlands.
Because Komineft, the company that owned the 20-year old pipeline has a history of accidents caused by age and corrosion, they were fined $600,000 for its pipeline spill, although the company was unable to pay much of the amount owing due to financial challenges.[47]

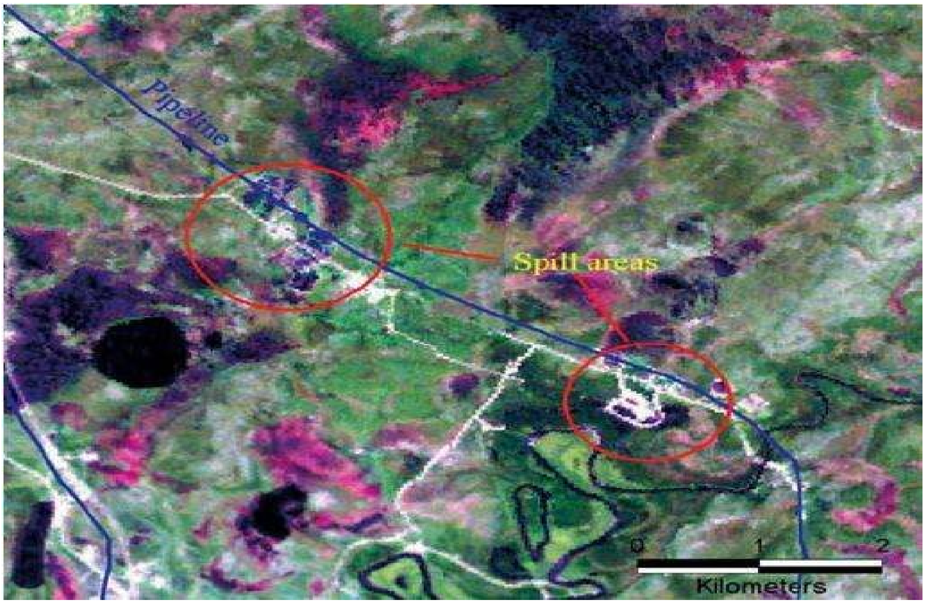

Figure 9 The circled parts are the Oil spill affected areas from 1994 close to the Kolva River, visible in the Landsat satellite image acquired on 24 June 2000, channels RGB 1⁄4 2, 5, 1. Blue line indicates oil pipelines. Some dams can be spotted.

The Ixtoc 1 Oil Spill

In the early hours of June 2, 1979, the Ixtoc well in the Gulf of Mexico started to lose drilling mud, and there was loss of circulation in the well. But several attempts made to regain circulation in the well were unsuccessful.[48] Then at about 3:30 AM, the well exploded, caught fire, and destroyed the platform, causing gas and oil to leak from the sight. PEMEX (Petróleos Mexicanos), Mexican Petroleum Company decided to drill two relief wells as a solution to stop the dispersal of the oil. Unfortunately, those two wells did very little to stop the spread.[49]
During the 290 days that the well remained uncapped, 475,000 metric tonnes of oil (3.4 million barrels) leaked into the Gulf of Mexico.[48,49] This is as shown in Fig. 5. 72% of the spilled oil evaporated or sank to the sea floor, 6% washed ashore, 3% drifted to the beaches in the U.S., 12% biodegraded, and about 7% was recovered from the site.[49]

Figure 10 Ixtoc I oil well releasing thousands of metric tonnes of oil into the gulf. Source: Wikipedia Commons.

BP's Deepwater Horizon Oil Spill

The Deepwater Horizon (DWH) accident, one of the most famous oil spills occurred in the Gulf of Mexico in the year 2010 during the drilling rig explosion. During this disaster, more than 700 thousand tons of crude oil was spilled into Gulf of Mexico.[38] This accident reduced the biodiversity of the vertebrates and some aquatic lives. The cleaning cost of this spill was estimated to be 10 billion USD.[50] Reports in early 2012 indicated that the well site was still leaking.[51,52] The U.S. federal government estimated the total discharge at 4.9 million barrels (210 million US gal; 780,000 m3).[33] Deepwater Horizon oil spill is regarded as one of the largest environmental disasters in American history and can be called the worst oil accident in history.[26,53] It is also regarded as the largest marine oil spill in the history of the petroleum industry and estimated to be 8 percent to 31 percent larger in volume than the previous largest, the Ixtoc I oil spill that occurred in the Gulf of Mexico. Some notable oil spill cases around the world are shown in table 1 below.

Oil Spill	**Location**	**Date**	**Barrels of Crude Oil Released (thousands)**
Kuwaiti Oil fires	Kuwait	Jan., 16, 1991- Nov., 1991.[33]	1,000,000
Lakeview Gusher	California, USA	March 14, 1910- Sept., 1911.[33]	9000

Castillo de Beliver	South Africa	August 6, 1983.[33]	1,848
Funiwa-5 oil well blowout	Niger Delta region, Nigeria	Jan., 17, 1980.[54, 55]	. 40,000
ABT Summer	Angola	May 28, 1991.[33]	1,907

Table 1: Some notable Oil Spill Cases around the world

Remediation Approaches for Crude Oil/Petroleum Spill

The faster the response to oil spill, the more chances it is to be prevented and stopped from contaminating the environment.[56] The objective of oil spill response is in mitigating its adverse impacts rather than monitoring the contaminants and allowing its natural attenuation. More so, the first aim of a respond to oil spill is controlling the source and preventing the oil spread. The response could be any strategy, method, technology, or equipment to control the spill and remediate its negative effects. In addition to the fast response, stewardship is necessary to monitor and oversee the movement of oil. Hence, remediation is the process of bringing back the natural status of soil, water or air that existed prior to a contamination by degrading and transforming the contaminants into less harmful and even harmless forms. When it is not possible, treatment is done by reduction of contaminants mobility and migration to prevent their spreading into uncontaminated areas. With this approach, the contaminant toxicity does not change, but the chances of their further distribution to the environment though absorption and dilution are minimized. Several remediation and responses to the oil spills are available which include physical, chemical, and biological methods.[25,35,57,58] These techniques or methods depend on the media (i.e air, water and soil) and the contaminant (e.g. heavy metal, PCB).[59] Furthermore, the type of oil, location and size of spill, local regulation and standards are put into consideration in the choice of the best clean-up approach. It is important to know that for selecting the best methods, other various criteria including efficiency, time, cost, reliability, effect on oil characteristic, and necessity of the post-remediation treatment of the applied method must be considered as well.[60] The use of mechanical equipment for spill removal such as skimmers, booms, barriers and sorbents, dispersants and controlled in-situ burning are among the response strategies.[61] A combination of biological, chemical and physical processes, such as incineration, use of landfills, solidification and biological treatments can be used to treat hydrocarbon contaminated soils.[62-64]

Physical/Mechanical and Chemical Approaches

Mechanical and chemical methods are common conventional technologies used in treating contaminated soils. While physical/mechanical method principally involves soil replacement and thermal desorption, the chemical method involves washing contaminated soil using clean water, reagents, and solvents that can leach the contaminants from the soil.[35,58,65] The commonly used mechanical methods are: booming and skimming, manual removal (wiping), mechanical removal, water flushing, sediment relocation and tilling.[66] The chemical method could be achieved through chemical leaching, chemical fixation, electrokinetic remediation, vitrify technology, chemical immobilisation, oxidation, chemical fluid extraction and photodegradation among others.

While oil spill removal in the sea is done conventionally by using booms, skimmers, and big sponges as sorbents, vacuuming, in situ burning, and chemical dispersants. [26,60] On the other hand, oil spill in soil is removed using available methods such as solvent extraction, chemical oxidation, electrokinetic movement of contaminants, thermal desorption, flotation, washing with cosolvents or surfactants, chemical agents for oxidation-reduction, physical removal such as ultrasonication, excavation of soil and sediment or groundwater pumping.[35,67] These physical and chemical methods relying on incineration, volatilization or immobilization of pollutants are often adopted when the oil concentration is quite high at the contaminated sites.

In spite of the fact that these methods are often very effective in removing the original contaminants, there is, however, increasing disagreement over the use of these methods as they are capable of transferring the pollutants or produce secondary pollution which might pose long-term threat to the environment.[66,68,69]

Specifically, the chemical method is fast at cleaning-up of contaminants, but the major drawback is that the harmful wastes generated in the process include carbon dioxide and other greenhouse gases which are emitted into the surrounding environment.[70] In addition, the chemical method is expensive and has the potential to contaminate other environmental media including air and water bodies through the introduction of solvents and reagents during remediation.[58]

Biological Remediation Approaches

The biological methods (e.g. bioremediation and phytoremediation) have been of great research interest in the last decade owing to their various advantages. The aim of these biological and other forms of treatment is to reduce the level of hydrocarbon contaminants so that the soil can be either be safely re-used for other purposes or disposed off in landfill sites. Biological remediation is often used in the moderate oil-contaminated soils. Biological remediation is considered as a complementary treatment after conventional clean-up.[71] When compared to the mechanical and chemical remediation, biological remediation is regarded as a better remedial technology in soil contamination.

It is scientifically defined as detoxification, cleaning-up or transformation of pollutants or contaminants into less harmful forms by the use of biological agents. Biological remediation presents a potentially low-technology, cheaper option than physicochemical strategies.[68,72] First, they are more cost effective with little investment in labour. Second, they are more eco-friendly as the risk of causing secondary pollution is minimized. Third, they are applied for treatment of a wide range of environmental contaminants. Last but not the least; they can give an aesthetical value by providing pleasant landscape as in the case of phytoremediation.[11]

There are four types of biological methods that can be used in soil remediation: First, Bioremediation- the use of microorganisms (e.g. fungi or bacteria) to decompose organic pollutants; Second, Phytoremediation- the use of plants, especially the fast growing plants with large biomass; Third, the use of soil animals (e.g. earthworms) to accumulate, stabilize or breakdown the non-degradable contaminants in their body or in the soil; Fourth, the combined use of all organisms or even the combinative use of both physicochemical and biological methods.

In this review, concise information on the bioremediation approach has been provided.

Bioremediation Approach

Bioremediation is an approach that uses biological agents such as microorganisms to reduce or break down hazardous organic materials to harmless compounds in order to restore the natural status of contaminated sites. In this approach, microorganisms use contaminants, especially petroleum hydrocarbons as substrates to produce biomass and break down pollutants into water carbon dioxides, and other harmless compounds such as fatty acids.[60,73,74] Research has proven that auxiliary treatments such as aeration and temperature adjustment can improve the bioremediation process.[35] In the course of bioremediation, petroleum hydrocarbons are used either as growth medium or as cometabolism. This signifies that the contaminants can be considered as carbon and energy sources and are totally biodegraded and mineralized or utilized as extra nutrition source in combination to growth substrate.[75] However, the ability of microorganisms in degrading petroleum hydrocarbons greatly depends on available chemical compounds and environment conditions.[25] This environmental strategy or technique was developed in the 1940s and has been carried out on different oil spill sites till today. For instance, in 1994, the effectiveness of bioremediation strategy for the removal of weathered crude from contaminated sand was seen in Exxon Valdaz oil spill in La Touche Island. The results indicated 70% of the semivolatile components were removed from the sample.[76] In addition, in the case of oil spill in Brittany coastline, it was reported that bioremediation was a fast and effective method used in containing the spill.

Bioremediation can be carried out in situ or ex situ processes.[75] In ex situ process, the contaminated material extracted elsewhere is treated, while in in-situ process, the treatment of contaminated matrix is done at the place of contamination.[67] These processes have been successfully and effectively used in the bioremediation of contaminated soil.[35] However, in-situ approach is more economical and safer than ex-situ with minimal disruption of the polluted environment.[75]

Whenever bioremediation is to be used as a treatment option to oil spills, the primary aim is addition of materials to the contaminated environment in order to accelerate the natural biodegradation process. For instance, the supply of nutrients enhances indigenous organism's growth and activity. Another means is exposure of the contaminated environment to non-indigenous microorganisms with enhanced capacity for hydrocarbon degradation. It is important to ensure that added materials (microorganisms and nutrients) reach the polluted region when bioremediation is to be utilized in the treatment of buried oil.[77] Reports have it that emulsion formation which is resistant to biodegradation can hinder the bioremediation process, because the microorganisms may colonize on the surface of emulsion, hence, making it difficult for microorganisms to reach the mass of emulsion.[37] It is worthy of note that the limiting factors for bioremediation process in marine environment are mainly a-biotic factors (e.g., oxygen, phosphate, and nitrogen concentration). However, for the bioremediation of contaminated soil, the limiting factors are usually aged spill, complexity of the soil matrix type, and nature of the contaminants.[78]

Any research on the bioremediation of oil spills must put into consideration; effects of environmental factors, metabolic pathways, hydrocarbon breakdown as substrate from genetic view point, in addition to the effects of hydrocarbon contaminants on microorganisms. The primary objective of this study was borne out of the need for monitoring the fate of hydrocarbon contaminants in the environment and the search for the novel strategy in accelerating the natural degrading process in order to overcome the rate-limiting factors.[25] Accelerating methods involve addition of microbes with higher oil-degrading ability or nutrients such as nitrogen and phosphorous.[60] For a bioremediation process to be effective, the presence of microorganisms with excellent physiological characteristics, enzymatic abilities, proper activity and growth conditions and bioavailable active microbial consortia play an important role.[75]

The several distinctive approaches adopted in the context of bioremediation are, Natural attenuation, bioaugmentation, and the combined biostimulation and bioventilation approach.[64,80-85] These approaches are briefly explained below.

Natural Attenuation

Natural attenuation is a series of physical, chemical or biological processes that act without human intervention to reduce the toxicity, mass, mobility, concentration or volume of contaminants in soil or groundwater, under favourable conditions.[86] Natural attenuation focuses on a careful monitoring of the natural rate of pollutant degradation, without any direct intervention.[87] In natural attenuation, pollutants are transformed into less harmful substances courtesy of a series of processes such as biodegradation, evaporation, dilution, volatilisation, dispersion, precipitation, sorption, etc.[88-90] This approach is called "monitored natural attenuation" when the process monitored, which may be utilized singly or in combination with other methods.[59]

Bioaugmentation

Bioaugmentation is process used to enhance the performance of the microbial population through the addition of bacterial with specific catabolic activities, strains or enrichment consortia to increase the rate of contaminant degradation.[35,64,80]

This approach is considered when the native microbial populations are not enough or not capable of degrading the pollutant mixtures such as petroleum.[91] For example, polynuclear aromatic hydrocarbons are usually hard to be degraded.[71] More so, microorganisms with enhanced biodegradation ability are added to the polluted environment to supplement the naturally available microbes using this approach.

There are varied methods available for this approach. Commonly, the nonindigenous microbes from other polluted environments are added to the target site.[71] This approach is known as "Genetic Bioaugmentation (ie the process of obtaining enhanced activity from an introduced donor organism into a member of the indigenous soil population after gene transfer.[92] Alternatively, microorganisms from the target site are separated, mass cultured under laboratory conditions in bioreactors and inoculated at the target site. This process is known as *autochthonous bioaugmentation or cell bioaugumentation (*ie the inoculation of given soils with microorganisms either being pure or mixed cultures characterized with desired catabolic potentials and used to describe the cases where the bioaugmentation is carried out by the native microorganisms of the contaminated site after enrichment and are reapplied to the site.[35,93] But for successful implantation of bioaugmentation, inoculated microorganisms must have the ability to:

(i) survive and proliferate in the soil;
(ii) have direct contact with the pollutant, which must be in a bioavailable form
(iii) express in-situ their capacity to metabolise the pollutant at a reasonable rate. Seeding of microorganisms to the contaminated site can minimize the lag period for starting biodegradation process. Adaptation problem is avoided when the seeding is completed by the enhanced native organisms taken from the target site.[71] The criteria for selection of the microbes are on their physiology and metabolic ability.[35] More so, the addition of native microbial strains can have an impact on indigenous microorganism population, and the essential processes they carry out.[94]

Bioaugmentation using bacterial consortia, based on cross-feeding interactions, is commonly introduced where the biochemical steps in the biodegradation pathway are shared among community members.[95] One merit of using a consortium of several bacterial strains is that potentially toxic intermediate products generated by one strain may be degraded by another strain.[96] One drawback to this approach is that there is no single strain of bacteria that has the requisite metabolic ability to degrade all oil components. Thus, studies recommend diverse types of bacteria strains and fungi for the remediation of hydrocarbon contaminants.

Biostimulation

Biostimulation is the bioremediation mechanism that involves the adjustment of the environmental parameters (e.g. temperature, moisture, redox potential, aeration, pH and), the introduction of nutrients (e.g. nitrogen, phosphorus) and electron donors and acceptors to contaminated or polluted soil, in order to stimulate or enhance the growth of oil-degrading microbial populations and, thus, to decrease the concentration of soil pollutants. Electrons are added to enhance the oxidation and reduction mechanism. The primary advantage of this bioremediation approach is the fact that pollutant degradation is carried out by indigenous microbial populations present in the soil ecosystem. This strategy which primarily focuses on the addition of nitrogen (N) and phosphorus (P) nutrients has been used extensively for the bioremediation hydrocarbon polluted soils, as the biodegradation of these compounds results in a fast depletion of the available inorganic nutrients, such as N and P.[97]

In marine environment, addition of Nitrogen and Phosphorous is quite difficult. Therefore, uric acid (waste product of animals (birds, reptiles, insects, etc.) is added instead. Uric acid, having low water solubility can attach to petroleum hydrocarbons, hence, can be utilized by bacteria as nitrogen source or both nitrogen and carbon source.[98] Researchers have observed that for the degradation of light crude oil, addition of nitrate is more effective than ammonia in seawater, whereas in the salt-marsh soil, the reverse is the case. Fortunately, no negative impact, such as algal blooms was seen when nitrogen was added.[71]

Good results were obtained when this approach was used on sediments of the contaminated sea and shoreline after Exxon Valdez spill in Alaska, and the rate of biodegradation increased three to five times by addition of iron, phosphorus and nitrogen as fertilizers.[73]

Bioventilation

Bioventilation involves the addition of oxygen to soil voids so as to stimulate the growth of microbes. Bioventing enhances the activity of indigenous bacteria and archaea and stimulates the natural in-situ biodegradation of hydrocarbons by inducing air or oxygen flow into the unsaturated zone and, if necessary, by adding nutrients.[99]

Oxygen is a necessity and often the limiting factor in the process of biodegradation and in fact general plant growth, as it enhances microbial metabolism of organic matter and generate more energy. During bioventing, oxygen may be supplied through direct air injection into residual contamination in soil. Bioventing primarily assists in the degradation of adsorbed fuel residuals, but also helps in the degradation of volatile organic compounds (VOCs) as vapors move slowly through biologically active soil.[100] Compared to natural attenuation, bioventing has been shown to be effective and efficient in the remediation of a blend of diesel and biodiesel fuel with increased remediation rate.[101] This method has recorded 85% efficiency in contaminant degradation, compared to 64% seen in natural attenuation after 60 days.[35] The bioventing approach is of different forms; conventional, soil vapor vacuum extraction and the passive forms. In conventional bioventing systems, oxygen is delivered by an electric blower to subsurface wells, unlike in soil vapor vacuum extraction, where bioventing uses low airflow rates to provide only enough oxygen to sustain microbial activity. The passive bioventing systems however, use natural air exchange to supply oxygen to the subsurface via bioventing wells. A one-way valve, installed on a vent well, allows air to enter the well when the atmospheric pressure is higher than the pressure inside the well. When atmospheric pressure drops (due to a change in barometric pressure) below the subsurface pressure, the valve closes, trapping the air in the well and increasing oxygen to the soil surrounding the well.

It is worthy of note that the bioventing approach presents some limitations and concerns. First, bioventing performance reduces when the soil contains high moisture or has low permeability. Extremely low soil moisture content may limit biodegradation and the effectiveness of bioventing. Second, low temperature may slow bioventing remediation process. Third, vapours can build up in basements within the radius of air-injection well influence.

However, the extraction of air near the structure can alleviate this problem. Furthermore, saturated soil lenses are difficult to aerate and fluctuating water tables create saturated soil zones of low air permeability. More so, the monitoring of vapour at the soil surface may be required.
Bioventing is considered a commercial technology and has been approved and adopted by Environmental Protection Agencies (EPAs).

Combination of Biostimulation and Bioaugmentation

The physicochemical conditions (temperature, pressure, pollutant surface area, oxygen content, nutrient availability, pH, salinity, oil composition, etc.) have been known to influence the natural bioremediation process. Therefore, when applying bioremediation as a response to the oil spill, three main approaches are available which are biostimulation (enhancing the nutrients availability, mostly nitrogen and phosphorus to initiate the growth and accelerate the biodegradation process), bioaugmentation (inoculation of microorganisms with enhanced ability to degrade petroleum hydrocarbons in order to facilitate the process) and bioventilation (addition of oxygen to soil void to stimulate the growth of microbes). It was reported that the effects of bioaugmentation can be observed much faster than biostimulation.[77] However, the most promising approach is the combinative application of biostimulation and bioaugmentation methods in addition with biosurfactants.[83,84,98]

Factors Influencing the Rate of Bioremediation

Several factors have been found to influence Bioremediation. These are biological and environmental factors. Biological factors (choice of microbes, metabolic parameters, mass transfer parameters and bioavailability) and environmental factors (Nutrient, temperature, moisture content, oxygen, pressure, soil pH, detergent, salinity, contaminant surface area, heavy metal, type of soil, contaminant nature and composition) have major effects on bioremediation. Most of the factors can be manipulated to accelerate the rate of natural biodegradation, while factors such as salinity are not adjustable in real field.[71] Among the biological factors, metabolic parameters, mass transfer parameters in cell membrane and bioavailability must be put into consideration.[98] The effects of some of these factors are explained as follows.

Microorganisms

Although, many microorganisms can degrade the crude oil present in the soil, it has been found rewarding to employ mix culture as opposed to pure cultures in bioremediation since it shows the synergistic interaction.[102] More so, studies have shown that indigenous microbes have the capacity of adapting and responding rapidly to contamination under favorable nutrient conditions and degrading contamination.[28,103] Even in extreme environmental conditions (e.g. deserts), microbes have the capacity of degrading hydrocarbon contamination.[103] The process of using indigenous microbes in degrading soil contaminants is known as biosparging, and it is regarded as the most non-invasive and cost efficient.[68] Bacteria such as Rhodococcus, Acinetobacter, Pseudomonas (e.g. P. cepacea, P. fluorenscens and P. putida), Mycobacterium, Arthrobacter, Acaligenes and Acidobacteria species and some fungi such as Pencillium, Mucor and Aspergillus have been found capable of degrading hydrocarbons.[104,105]

Temperature

Among all the environmental factors, temperature is the most important one that determines the survival of microorganisms and hydrocarbon composition.[106] In cold environments such as the Arctic region, oil degradation through natural processes is very slow which puts the microbes under severe pressure in cleaning up spilled petroleum. Temperature also speeds up or slows down bioremediation process because it influences microbial physiological properties. The sub-zero temperature of water in Arctic region causes the transport channels within the microbial cells to stop functioning, and this may even freeze the entire cytoplasm, thereby rendering most oleophilic microorganisms metabolically inactive.[107,108] The rate of microbial activities increases with temperature and reaches its maximum level known as an optimum temperature. It declines sharply with further increase or decrease in temperature and eventually stops after reaching a specific temperature. In all ranges of temperature, biodegradation can take place at an optimum temperature between 20-30 ^{o}C.[109] However, the rate decreases with decrease in temperature. In addition, temperature impacts viscosity and consequently toxicity; because at higher viscosity, the toxic light hydrocarbons are less volatile. Furthermore, low temperature results in higher viscosity, lower volatile evaporation rate and higher water solubility of the oil which can delay the biodegradation process. More so, the solubility of petroleum hydrocarbons changes with temperature. At low temperatures, alkanes with shorter chains are more soluble, while higher temperatures favours the solubility of many light aromatics. In several cases such as arctic and subarctic regions, bioremediation of oil spill was successfully done.[110]

Oxygen

Despite the fact that oxygen is the rate limiting factor, it is still one of the most essential elements of microbial degradation of hydrocarbons. Oxygen is vital for the initial breakdown of hydrocarbons and succeeding reaction may need it. Oxygen availability depends on water and wave turbulence, physical state of oil and substrate availability. In the presence of oxygen, complete degradation of oil takes place. Dissolved oxygen is required for degradation and oxidization of the pollutants. Usually, there is no limitation of oxygen on superficial water in the sea and freshwater. However, oxygen may be lacking in some subsurface sediment such as anoxic zones in water columns. Freshwater wetlands, tidal flats, dense marine shorelines, coastal salt marshes, and bottom layer of soil are other examples of the environment with limited oxygen. In order to facilitate the biodegradation rate in a better way, some organisms require oxygen while others do not. Biological degradation is done in aerobic and anaerobic conditions, because oxygen is a gaseous requirement for most living organisms. The presence of oxygen in most cases can enhance hydrocarbon metabolism.[107] However, reports have shown that anaerobic degradation of certain pollutants can take place in insignificant extent of oxygen.[111,112] The advantages of anaerobic system over aerobic system are utilization of less space and no energy requirements.[112]

Pressure

Pressure can impact the rate of bioremediation since at higher pressure, such as in the deep ocean, the rate of biodegradation decreases.

Surface Area of Contaminants

The surface area of contaminants can change the interface of oil and water. Rate of biodegradation is enhanced by increase of surface area of contaminants. For example, in marine environment, turbulence of the sea surface can impact bioremediation process by influencing dispersion. This causes dilution of the available nutrients, and the spread of the oil.[71] More so, the degree of spreading can affect the surface area. Hence, oil spreads and forms a thin slick in aquatic system.[113]

Soil pH

The soil pH which is the degree of its acidity, basicity and alkalinity, has its own impact on microbial metabolic activity and also increases and decreases oil removal process. The measurement of pH in soil could be an indicative of the potential for microbial growth.[114] Too high or too low pH values have not yielded positive results and the metabolic processes are highly influenced by even slight changes in pH.[115] At higher pH, the rate of hydrocarbon degradation increases. Biodegradation can occur under a wide range of pH. However, a pH of 6.5 to 8.5 is generally the optimum for biodegradation in most aquatic and terrestrial systems.[109]

Nutrients

The presence of nutrients including nitrogen and phosphorous is a more limiting factor than oxygen. Carbon is one of the basic forms of nutrient required for living organism. In addition to this, the bacteria also need macronutrient like nitrogen and phosphorous to ensure effective degradation of the oil. The nutrients are consumed not only by pollutant-degrading microorganism but also other microorganism such as phytoplankton. Precipitation of phosphorus may also compete with oil-degrading microorganism. Microorganisms are present in contaminated soil but not in the numbers required for bioremediation to occur. Therefore, their growth and activity need to be stimulated.

Moisture

Moisture influences the rate of contaminant metabolism because it influences the type and amount of soluble materials that are readily available as well as the osmotic pressure and pH of terrestrial and aquatic habitats.[116]

Contaminants nature/toxicity

The adaptability of the microorganisms and their resistance to extreme environmental contaminants are an important factor in bioremediation process.[24] However, even adapted microorganisms are not effective for biodegradation of extremely high amount of pollution. For instance, earthworms cannot survive in the soil in which oil content contamination is more than 3%. At high concentrations of toxicity, contaminants can create toxic effects to microorganisms and slow down bioremediation. The extent and mechanisms of toxicity differ with specific pollutants, their concentration, and the exposed microorganisms. Some organic and inorganic substances are toxic to targeted life forms.[117] At oil level of 1%, almost 100% inhibition of bacterial activity was observed.[35] During oil spills, the concentration of petroleum hydrocarbons is usually far more than tolerable limits.[113]

Detergent

Oils are hydrophobic in nature. Their limited availability to bacteria leads to the slow degradation. Addition of detergent to the oil contaminated soil helps in desorbing the hydrocarbon, and hence boosts up the bioremediation process. Generally, microbiologically produced detergents called rhamnolipids are used.[118]

Heavy Metals

Heavy Metals are important in small amount for bacteria and fungus, but in high quantity, they inhibit the metabolic activity of the cells. Metal compounds have direct and indirect impact on rate of degradation.

Some of the environmental factors requirements for effective degradation of crude oil contaminants are presented in the table 2 below

Parameters	**Requirement for microbial activity**	**Optimum value for an oil degradation**
Temperature (°C)	15–45	20–30
Soil pH	5.5–8.8	6.5–8.0
Nutrient content	N and P for microbial growth	C:N:P = 100:10:1
Oxygen content	Aerobic, minimum air-filled pore space of 10%	10–40%
Contaminants nature and composition	Not too toxic	Hydrocarbon 5–10% of dry weight of soil
Heavy metals Concentration (ppm)	Total content 2000	700
Type of soil	Low clay or silt content	NAD
Soil moisture	25–28% of water holding capacity	30–90%

Table 2: The Optimum environmental condition for degradation of crude oil contaminants.[109]
NAD= No Available Data

Advantages and Disadvantages in Bioremediation Strategy

Bioremediation, being of the remediation techniques have some advantages and disadvantages which are briefly explained below.

Although detailed economic analysis of the bioremediation process has not yet been properly done, bioremediation method is a cost-effective one.[71] It has no significant adverse effects such as production of secondary contaminants, minimal physical disruption of the site, effectiveness in removing toxic compounds and simpler mechanical technologies.[20]

In spite of the advantages of Bioremediation, the process is slow and requires weeks or months for effective clean-up and in the case of highly polluted environment, the process is less effective.[119] Furthermore, it is a less effective treatment strategy in the sea and the available knowledge is still rough and mainly focused on the application of prokaryotic organisms.[24]

Bioremediation Microorganisms for Bioremediation

According to scientific reports, more than 200 different species of bacteria, fungi, and yeasts have the capacity to degrade petroleum hydrocarbons. These microorganisms are present naturally in freshwater, marine and soil. The biodegradable petroleum hydrocarbon compounds range from methane to C_{40}. For the purpose of classification, almost 79 bacterial, 9 *cyanobacterial genera*, 103 fungi, 14 algae, and 56 yeasts have the ability of degrading the hydrocarbon pollutants.[71,98]

Several groups of indigenous soil bacteria are able to degrade different petroleum hydrocarbon compounds. These bacteria include; *Pseudomonas* strains (isolated from soil and aquifers to degrade polycyclic aromatic hydrocarbons (PAHs)].[37] Other microorganisms with petroleum hydrocarbons degrading capacity are; *Acinetobacter* sp., *Yokenella* sp., *Alcaligenes* sp., *Alcanivorax* sp., *Microbulbifer* sp., *Stenotrophomonas* sp., *Sphingomonas* sp., *Sphingobacterium* sp., *Micrococcus* sp., *Cellulomonus* sp., *Dietzia* sp., *Roseomonas* sp., *Gordonia* sp., *Corynebacterium* sp., *Flavobacter* sp., *Streptococcus* sp., *Providencia* sp., *Capnocytophaga* sp., *Bacillus* sp., *Moraxella* sp. and *Enterobacter* sp.[25]

Alcanivorax sp. bacteria and *Cycloclasticus* sp. can respectively use aliphatic and aromatic hydrocarbons, as their carbon source. Some bacteria can produce biosurfactants which enhance bioremediation by reducing surface tension and increasing crude oil uptake. However, factors such as availability of nutrients and nature of oil contaminants have a great influence in petroleum hydrocarbons degradation.[24]

Some fungi possess the ability of degrading petroleum hydrocarbons. However, they need longer period for thorough and effective degradation. Fungus belonging to, *Amorphoteca* sp., *Graphium* sp., *Neosartorya* sp., *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., *Talaromyces* sp., and *Paecilomyces* sp., are among the microorganisms with the capacity to degrade petroleum hydrocarbon compounds. Report has it that, White rot fungi has all it takes to degrade compounds such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs).[120] Another report revealed that some yeasts including *Candida* sp., *Pichia* sp., and *Yarrowia* sp., have the potential to degrade the organic compounds available in oil contaminants.[25] More so, some researchers are of the view that in some particular occasions, fungi degrade petroleum better than bacteria. However, there is limited available information regarding fungal bioremediation of marine contaminated sites.[24]

The use of indigenous microorganisms available in a polluted site is considered to be a promising method for bioremediation of petroleum hydrocarbon contaminants, because these native microorganisms can easily adapt to the prevailing environmental conditions. However, microorganisms with enzymatic capacity for pollutant degradation may be absent which leads to longer process. In the research work performed by scholars, fungal community having the potential of degrading oil spills were isolated from sediments (17 strains) and Mediterranean marine (67 strains).[24] The fungal growth was enhanced by crude oil which served as the carbon source. Among them *P. citreonigrum*, *A. terreus*, and *T. harzianum*, produced the highest dichlorization percentage and *A. terreus* presented the maximum yield in degrading hydrocarbon compounds.[24]

It should be noted that whenever microorganism communities are exposed to hydrocarbons (contaminants), they adapt gradually and undergo selective genetic enrichment. After adaptation, the bacteria population with hydrocarbon degrading potentials and plasmids of the bacterial cells that encode hydrocarbon catabolic genes increases.[75] The increase in the population of oil-degrading microorganisms has been observed for *Cycloclasticus pugetii* and *Alcanivorax* sp.[98]

Genetically Modified or Genetically Engineered Microorganisms [GMMs or GEMs]

A genetically engineered microorganism (GEM) or modified microorganism (GMM) is a microorganism whose genetic material has been altered using genetic engineering techniques inspired by natural genetic exchange between microorganisms. These techniques are generally known as recombinant DNA technology. Immediately, the prospect of releasing genetically modified microorganisms for bioremediation became a reality, much of the research effort in the field was aimed at bio-safety and risk assessment.[116] Genetically engineered microorganisms (GEMs) have shown potential for bioremediation of soil, groundwater and activated sludge, exhibiting the enhanced degrading capabilities of a wide range of chemical contaminants.[121]

Superbug, the first genetically engineered microorganism (GEM) was built in 1970 and was able to degrade oil. This was done with plasmid transfer in utilizing some toxic hydrocarbons including hexane, octane, toluene, xylene, camphor, and naphthalene.[122] The development of genetically engineered microorganisms (GEMs) became more relevant in the early 1980s after improvement was made on genetic engineering methods and via research on metabolic abilities of microorganisms. It was in 1981 that the first two genetically modified strains were patented. These two strains are *Pseudomonas putida* (NRRL B-5473) and *Pseudomonas aeruginosa* (NRRL B-5472) containing genes that offer them the capacity for degrading camphor, salicylate and naphthalene. The two operons available in these strains are responsible for metabolism of m-xylene, toluene and *m*-ethyltoluene.

One of the limitations in the use of microorganisms in bioremediation is the toxicity of some of organic pollutants on microorganisms in addition to the complexity caused by diversity of contaminants. This is more severe about new anthropogenic contaminants released into the environment, since the microorganisms have not evolved the right catabolic pathway for the degradation of contaminants within a short time.[123] Thus, the primary focus of genetic engineering and manipulation of microorganism for bioaugmentation with genetically engineered microorganism process. Recent breakthrough in molecular biology promoted this area of research (ie GEMs) for specific bioremediation.

During genetic modification, microorganisms are fortified with new genetic properties so as to have the ability in biodegrading specific pollutants that are not degraded by natural microorganisms, properly and speedily. Proper microbiological information in addition to knowledge on ecological and biochemical mechanisms is required for combination of various desirable metabolic characteristics of organisms, and the manipulation of vital genetic properties.[124] In order to build genetically modified bacteria, prompt knowledge on the way bacteria break down molecules of petroleum compounds for the removal of oil spills is needed. The development of GEMs with enhanced ability for organic compounds biodegradation is feasible since the enzymes, degradative mechanism, and the vital genes are understood and biochemical reactions are thoroughly explained. These strategies are explained below.

There are four strategies employed in the GEM process. They are: identification of suitable microorganisms suitable for modification with relevant genes; pathway construction and regulation; modification of enzyme specificity and affinity and lastly bioprocess development, monitoring, and control, bio-affinity, bio-reporter sensor applications for chemical sensing, toxicity reduction and end point analysis.[122]

In the first approach, microorganisms are identified and selected based on their adaptability for survival in the environment as some microorganisms that can survive in the soil ecosystem may not be able to survive in the aquatic ecosystem and thus cannot be used successfully.

The second approach which is the pathway construction, extension and regulation is carried out through the enhancement of existing catabolic pathways or to extension of these pathways in degrading compounds which are not easily degraded using wild strain. The complete catabolic pathway may be encoded by a single microorganism, or by a consortium of microorganisms, each performing one or more of the stages of bioremediation. In this approach, constructed GEMs possess the degradation potentials of different microbial communities due to the alteration of gene sequences which further improves the efficiency and efficacy of the catabolic pathways.

In the third approach (ie modification of enzyme specificity and affinity), GEMs are built by the hybrid gene clusters which alter their enzymatic activity and enzyme substrate specificities. This process is performed owing the fact that each stage of the metabolic pathway is mediated by enzymes that are produced by the transcription and translation of specific genes. These gene clusters encode the enzyme possessing enhanced or improved transforming capability. For instance, E. coli strain is genetically engineered to possess a hybrid gene cluster for the degradation of trichloroethylene (TCE).

The fourth and last approach, which is the bioprocess development, monitoring, and control, and bioaffinity, bioreporter, sensor applications for chemical sensing, toxicity reduction, and end point analysis has insufficient information. However, this necessitated the development and use of the lux gene-based system that offers several advantages for monitoring bioremediation processes. Bioluminescence can be easily detected and do not require expensive devices, exogenous addition of chemicals or co-factors. Furthermore, GEMs possess chemical sensors that allow the monitoring of bioavailability of contaminants rather than just contaminant presence. Bioluminescence producing genetically engineered microorganisms also helps understanding the spread of microbes in the polluted area and end point of bioremediation process.[122] The drawbacks of this approach are; the survival of GEMs in the environment and its public acceptance, which hinder the wide application.

The figure 6 below depicts the processes involved in the bioremediation of contaminants/pollutants vis-à-vis, crude oil spill.

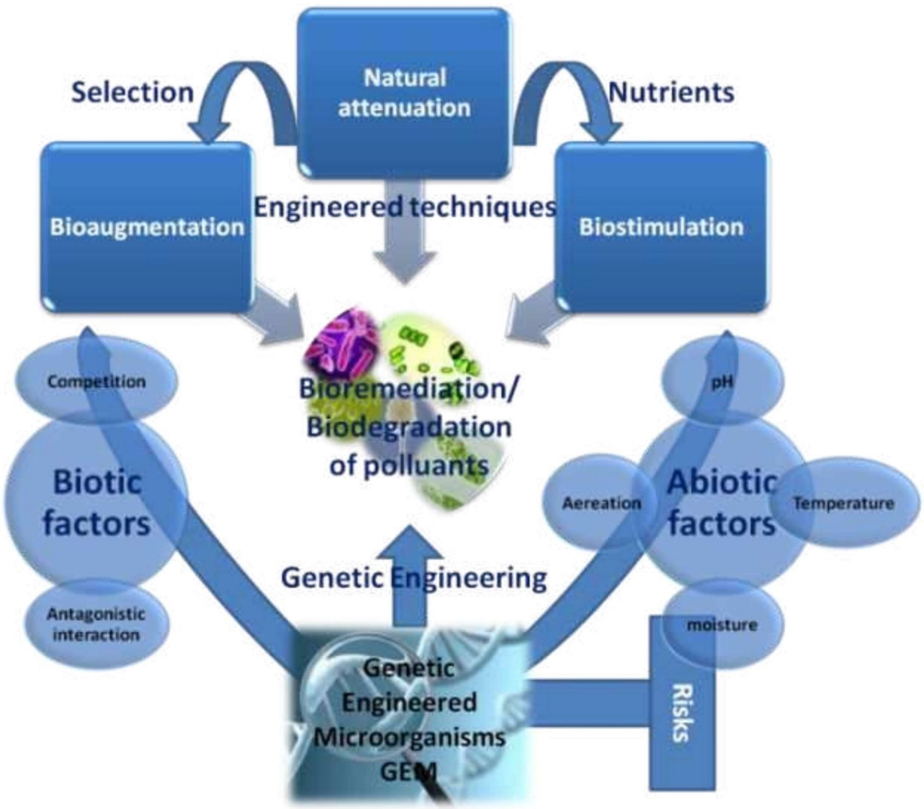

Figure 11 Process of Natural Attenuation

Bioremediation of pollutants using biodegradation abilities of microorganisms, a process that involves the natural attenuation, although it may be enhanced by engineered techniques, either by bioaugmentation or by biostimulation. Factors (Biotic and Abiotic) influencing the rate of biodegradation are shown as well.[125]

Conclusion and Recommendation

Land and water body contamination owing to oil spill has adverse effects on the terrestrial and aquatic ecosystems. There are several available remediation approaches for treatment of contaminated sites for their return to natural state. However, global trials of many of these approaches in the past have yielded little or no positive results owing to reasons ranging from soil type, environmental conditions to the consequences of the use of these methods on crude oil contaminated sites. While the remediation by physical remediation methods are labour intensive, expensive and not suitable for large scale contamination, the chemical remediation methods are expensive and could lead to contamination of other environmental media like air and water through the introduction of solvents and reagents during remediation. Following the success of field trials using bioremediation and the selection of remediation technologies currently in practice globally, evidence from researches and case studies, taking into consideration the constraints and advantages has shown that bioremediation is a suitable technology for the treatment of oil spill contamination. Obviously not all bioremediation methods will work in all instances of a spill due to location, and the grade of oil contamination, but with a list of viable options of species subject to the type of spill will advance the incorporation of this method. This approach is considered because of its public acceptance, relative zero greenhouse gas emission, environmental friendliness and lower labor cost of remediation. The combinative use of technologies is considerable; but, climatic factors and long-term environmental and socio-economic impacts should be the guiding principles for the choice of any other available options. Again, before total remediation is considered, treatability studies have to be carried out as bioremediation is a site-specific technology.

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References

1) Department for Environment, Food and Rural Affairs (DEFRA) of UnitedKingdom (2009). Soil strategy for England supporting evidence paper. Available at: http://archive.defra.gov.uk/environment/quality/land/soil/documents/evidence-paper.pdf.
2) Tinsley, A.; Farewell, T., (2015). Soil degradation: a growing concern. Dissecting Soil: Safeguarding an Invaluable Natural Resource. The Environmentalist, IEMA, UK: pp. 14–17.
3) Aisien, F. A.; Chiadikobi, J. C.; Aisien, E. T. (2009).Toxicity assessment of crude oil contaminated soil in Niger delta areas. Advanced Materials Research, 63: pp. 451 - 455.
4) Bossert, I.; Bartha, R. (1984). The fate of petroleum in soil ecosystems. In R.M. Atlas, ed., Petroleum Microbiology. New York: Macmillan.
5) Kaimi, E.; Mukaidani, T.; Tamaki, M. (2007). Screening of twelve plant species for phytoremediation of petroleum hydrocarbon-contaminated soil. Plant Prod. Sci.10: pp. 211–218.
6) Alkorta, I.; Garbisu, C. (2001). Phytoremediation of organic contaminants in soils. Bioresour. Technol. 79: pp. 273–276.

7) Aisien, F.A., Oboh, I.O.; Aisien, E.T. (2013). Phytotechnology - Remediation of Inorganic Contaminants In: Naser et al (Eds.) Phytotechnologies: Remediation of Environmental Contaminants, CRC Press, Florida, USA: pp.75 - 82.

8) Frick, C. M.; Farrell, R. E.; Germida, J. J. (1999). Assessment of Phytoremediation as an in situ technique for cleaning oil-contaminated sites; Petroleum Technology Alliance Canada, Calgary: http://www.rtdf.org/pub/phyto/phylinks.htm.

9) U. S. Environmental Protection Agency (USEPA) (2011b). Oil: Crude and Petroleum Products. 2011b. Available at: http://www.eia.gov/energyexplained/index.cfm?page=oil_home.

10) US Department of Energy Website

11) Canadian Council of Ministers of the Environment (CCME) (2001a). Canada-wide standards for petroleum hydrocarbons (PHC) in soil. Available at: http://www.ccme.ca/assets/pdf/phc_standard_1.0_e.pdf.

12) Kamath, R. ; Rentz, J. A. ; Schnoor, J. L. ; Alvarez P. J. J. (2007). Phytoremediation of hydrocarbon-contaminated soils: principles and applications, Department of Civil and Environmental Engineering, Seamans Center, University of Iowa, Iowa City, Iowa, U.S.A. – 52242.

13) Chaineau, C.H.; Morel, J.L.; Oudot, J. (1997). Phytotoxicity and plant uptake of fuel oil hydrocarbons. J Environ Qual 26: pp. 1478- 483.

14) Mueller, K.E.; Shann, J.R. (2006). PAH dissipation in spiked soil: Impacts of bioavailability, microbial activity, and trees. Chemosphere 64: pp. 1006-1014.

15) Drake, E. N. (1997). Phytoremediation of Aged Petroleum Hydrocarbons in Soil.IBC Phytoremediation Conference, June 18-19, 1997, Seattle, WA.

16) Potter, T.L.; Simmons, K.E. (1998). Composition of petroleum mixtures: Amherst, Mass., Amherst Scientific Publishers, Total petroleum hydrocarbon criteria working group series, vol. 2, p. 102.

17) Pilon – Smits, E.A.H. (2005). Phytoremediation. Annual Review of Plants Biology, 56: pp. 15 – 39.

18) McGill, W. B.; Rowell, M.J.; Westlake, D.W.S. (1981). Biochemistry, ecology, and microbiology of petroleum components in soil. In Soil Biochemistry, Vol. 3 (eds. Paul, E.A. and Ladd, J.N.), Marcel Dekker, New York: pp. 229–296.

19) Edwards, D. A.; Andriot, M.D.; , M.A.; Tummey, A.C.; Bevan, C.J.; Tveit, A.; Hayes, L.A.; Youngren, S.H.; Nakles, D.V. (1998). Development of fraction specific reference doses (RfDs) and reference concentrations (RfCs) for total petroleum hydrocarbons (TPH). Volume 4, Total Hydrocarbon Criteria Working Group Series. Amherst Scientific Publishers, Amherst, Massachusetts.

20) Singh O.V.; Jain, R.K. (2003).Phytoremediation of toxic aromatic pollutants from soil. Appl Microbiol Biot 63: pp. 128-135.

21) Cheng Y, Wang L, Faustorilla V, Megharaj M, Naidu R, Chen Z (2017). Integrated electrochemical treatment systems for facilitating the bioremediation of oil spill contaminated soil. Chemosphere 175: pp. 294–299.

22) U. S. Environmental Protection Agency Office of Research and Development (USEPA) (2007). Developing innovative solutions for oil spill cleanup. Land Research Program. Available at: http://www.epa.gov/research/npd/pdfs/lrp-factsheet_oil-spills.pdf.

23) Canadian Council of Ministers of the Environment (CCME) (2008). Canada-Wide Standard for Petroleum Hydrocarbons (PHC) in Soil: Scientific Rationale Supporting Technical Document Executive Summary. Available at: http://www.ccme.ca/assets/pdf/phc_sr_std_es_1.0_e.pdf.

24) Epps, A. V. (2006) Phytoremediation of Petroleum Hydrocarbons. U.S. Environmental Protection Agency. Available at: http://clu-in.org/download/studentpapers/A_Van_Epps-Final.pdf.

25) Bovio E, Giorgio G, Prigione V, Spina F, Denaro R, Yakimov M, Calogero R, Crisafi F, Cristina Varese G (2017). The culturable mycobiota of a Mediterranean marine site after an oil spill: isolation, identification and potential application in bioremediation. Sci. Total Environ 576: pp. 310–318.

26) Jain PK ; Gupta VK; Gaur RK; Lowry M; Jaroli DP; Chauhan UK (2011) Bioremediation of petroleum oil contaminated soil and water. Acad J Inc 5(1): pp. 1–26.

27) Ng YF; Ge L; Chan WK; Tan SN; Hong Yong JW; Tan TTY (2015) An environmentally friendly approach to treat oil spill: investigating the biodegradation of petro-diesel in the presence of different biodiesels. Fuel 139: pp. 523–528.

28) Nardini, E., Kisand, V.; Lettier i, T. 2010. Microbial Biodiversity and Molecular Approach. Aquatic microbial world and biodiversity: Molecular Approach to improve the knowledge. JRC Scientific and Technical Report. European commission. Joint Research Center. Institute for Environment and Sustainability. p. 49.

29) Agency for Toxic Substances and Disease Registry (ATSDR) (1999). Toxicological profile for total petroleum hydrocarbons (PHC). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Available at: http://www.atsdr.cdc.gov/ToxProfiles/tp123.pdf.

30) European Environmental Agency (EEA) (2007). Progress in management of contaminated sites (CSI 015) Available at: http://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites/progress-in-management-of-contaminated-1.

31) Burton, P.J.; Messier, C.; Weetman, G. F.; Prepas, E. E.; Adamowicz, W. L.; Tittler, R. (2003). The current state of boreal forestry and the drive for change. In

Towards Sustainable Management of the Boreal forest. NRC press, Ottawa, Ont., Canada. pp. 1-40.

32) Robertson, S. J.; McGill, W. B.; Massicotte, H. B.; Rutherford, P. M. (2007). Petroleum hydrocarbon contamination in boreal forest soils: a mycorrhizal ecosystems perspective. Biological Reviews 82: pp. 213-240.

33) Laura Moss (2018). The 13 Largest Oil Spills in History, Mother Nature Network. Retrieved 28 Dec., 2012 (www.treehugger.com) the-largest-oil-spills-in-history-4863988.

34) https://en.wkipdia.org/wiki/Oil_spill.

35) Patrick Barkham (2010). Oil Spills; Legacy of the Torrey Canyon.The Guardian via www.theguardian.com/environment/2010/jun/24/torrey-canyon-oil-spill-deepwater-bp.

36) Lim M.W; Von Lau E; Poh P.E. (2016). A comprehensive guide of remediation technologies for oil contaminated soil – present works and future directions. Mar Pollut Bull 109(1): pp.14–45. http://dx.doi.org/10.1016/j.

37) https://www.britannica.com/list/9-of-the-biggest-oil-spills-in-history.

38) Atlas RM (1995). Petroleum biodegradation and oil spill bioremediation. Mar Pollut Bull 31: pp. 178–182.

39) Mapelli F.; Scoma A.; Michoud G.; Aulenta F.; Boon N.; Borin S.; Kalogerakis N.; Daffonchio D (2017). Biotechnologies for marine oil spill cleanup: indissoluble ties with microorganisms. Trends Biotechnol 35(9): pp. 860–870.

40) Thomas C.G. (1991). The Apex Barges Spill, Galveston Bay, July 1990. International Oil Spill Conference Proceedings: March 1991, 1: pp. 291-297.

41) Michel, J. (1991). Gulf War Oil Spill," in Oil Spill Science and Technology, ed. by M. Fingas (Gulf Professional Publishing, 2010), p. 1127.

42) Hawley T.M., (1992). Against The Fires of Hell: The Environmental Disaster of the Gulf War (Harcourt Brace Jovanovich, 1992).

43) Barcelo; Damia (2014). The Handbook of Environmental Chemistry 27.Verlag Berlin Heidelberg: Springer P4. ISBN: 978-3-642-38475-2.

44) Telegraph, 10 Largest Oil Spills in History (n.d.) (2017). Retrieved 18 October,(2017), from web site: http://www.telegraph.co.uk/news/worldnews/australiaandthepacific/newzealand/8812598/10-largest-oil-spills-in-history.html.

45) en.wikipedia.org/wiki/ss_Atlantic_Empress.

46) Gillis; Carly (2011) "Atlantic Empress and Aegean Captain Oil Spill: A Brief History". Counter Spill. Retrieved 1 November 2012.

47) Soter; Tom (1979). "Supertankers Collide in Caribbean". Firehouse. Cygnus Business Media. Retrieved 1 November, 2012.

48) http://ogpnetwork.com/october-1994-the-kolva-river-oil-spill.

49) Jernelöv A.; Lindén O. (1981) "Ixtoc I: A Case Study Of The World's Largest Oil Spill," Ambio 10, 299 (1981).

50) Soto, L.A.; Alfonso V.B.; Sergio L.; Marcial L. L.; Alejandro Y.A (2014). The Environmental Legacy of the Ixtoc-I Oil Spill in Campeche Sound, Southwestern Gulf of Mexico," Front. Mar. Sci. 1, 57 (2014). https://doi.org/10.3389/fmars.2014.00057.

51) Alessandrello M.J.; Juárez Tomás M.S.; Raimondo E.E.; Vullo D.L.; Ferrero M.A. (2017). Petroleum oil removal by immobilized bacterial cells on polyurethane foam under different temperature conditions. Mar Pollut Bull 122(1–2): pp. 156–160.

52) Jamail, Dahr (2012). "BP settles while Macondo 'seeps'". Al Jazeera. Retrieved 1 June, 2012.

53) Rocky K. (2012). The Macondo Monkey on BP's Back". Huffington Post. 30 September 2011. Retrieved 1 June, 2012.

54) Weber, H.R. (2010). "Blown-out BP well finally killed at bottom of Gulf". Boston Globe. Associated Press. Retrieved 26 February, 2011.

55) Raji, A.O.Y.; Abejide, T.S. (2013). An Assessment of Environmental Problems Associated With Oil Pollution and Gas Flaring in the Niger Delta Region Nigeria, C. 1960s–2000. 3: pp. 48–62.

56) Chinweze, C.; Abiola-Oloke, G.; Jideani, C.(2012). Oil and gas resources management and environmental challenges in Nigeria. J. Environ. Sci. Eng. 1: 535–542.

57) Helmy Q.; Laksmono R.; Kardena E. (2015) Bioremediation of aged petroleum oil contaminated soil: from laboratory scale to full scale application. Proc Chem 14: pp. 326–333.

58) Khan, F.I.; Husain, T.; Hejazi, R., (2004). An overview and analysis of site remediation technologies. J. Environ. Manag. 71:95–122. http://dx.doi.org/10.1016/j.jenvman.2004.02.003.

59) Yao, Z., Li, J., Xie, H., Yu, C., (2012). Review on remediation technologies of soil contaminated by heavy metals. Procedia Environ. Sci. 16: pp. 722–729. http://dx.doi.org/10.1016/j. proenv.2012.10.099.

60) Gomes, H.I.; Dias-Ferreira, C.; Ribeiro, A.B., (2013). Overview of in situ and ex situ remediation technologies for PCB-contaminated soils and sediments and obstacles for full scale application. Sci. Total Environ. 445–446:237–260. http://dx.doi.org/10.1016/j.scitotenv.2012.11.098.

61) Marzan L.W.; Sultana T.; Mahbub Hasan M.; Akter Mina S.; Islam R.; Rakibuzzaman A.G.M.; Hassan K.I. (2017) Characterization of furnace oil bioremediation potential of hydrocarbonoclastic bacteria isolated from petroleum

contaminated sites of the Sundarbans, Bangladesh. J Genet Eng Biotechnol 15(1): pp. 103–113.

62) Walker A.H. (2017). Oil spills and risk perceptions. In: Oil spill science and technology. Elsevier, pp. 1–70.

63) Scragg A (2005). Bioremediation In: Environmental Biotechnology, New York, United States, Oxford University Press Inc.

64) Adams R.H.; Olán-Castro D.; Guzmán-Osorio F.J.; Diaz-Ramirez I.J. (2009). Relationship between geomorphology and contamination with weathered hydrocarbons in an old river levee/marsh association. Int. J. Environ. Sci. Tech. 6 (4): pp. 527-538.

65) Yousefi Kebria D.; Khodadadi A.; Ganjidoust H.; Badkoubi A.; Amoozegar M. A. (2009). Isolation and characterization of a novel native *Bacillus* strain capable of degrading diesel fuel. Int. J. Environ. Sci. Tech. 6 (3): pp. 435-442.

66) Hamby, D.M., (1996). Site remediation techniques supporting environmental restoration activities — a review. Sci. Total Environ. 191: pp. 203–224. http://dx.doi.org/10.1016/S0048-9697(96)05264-3.

67) Zhu, X.; Venosa, A. D.; Suidan, M. T. (2004). Literature review of the use of commercial bioremediation agents for cleanup of oil-contaminated estuarine environments. EPA/600/R-04/075. Available at: http://www.epa.gov/osweroe1/docs/oil/edu/litreviewbiormd.pdf.

68) Balba, M.T.; Al-Awadhi, N.; Al-Daher, R., (1998). Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. J. Microbiol. Methods 32:155–164. http://dx.doi.org/10.1016/S01677012(98)00020-7.

69) Shukla, K. P.; Nand K.S.; Shivesh S. (2010). Bioremediation: Developments, Current Practices and Perspectives. Genetic Engineering and Biotechnology Journal, Volume 2010: GEBJ-3.

70) U. S. Environmental protection agency (USEPA) (1999). Phytoremediation Resource Guide, EPA 542-B-99-003, solid waste and emergency response (5102G). Available at: http://clu-in.org/download/remed/phytoresgude.pdf.

71) Cappuyns, V., (2013). Environmental impacts of soil remediation activities: quantitative and qualitative tools applied on three case studies. J. Clean. Prod. 52: pp.145–154. http://dx.doi.org/10.1016/j.jclepro.2013.03.023.

72) Jafarinejad S. (2017). Oil-spill response. Petroleum waste treatment and pollution control. Elsevier, Oxford, pp. 117–148.

73) Obuekwe, C. O.; Al-Muttawa, E. M. (2001). Self-immobilized bacterial cultures with potential for application as ready-to-use seeds for petroleum bioremediation. Biotechnology Letters 23: pp. 1025–1032.

74) Martin C.W.; Hollis L.O.; Turner R.E. (2015). Effects of oil-contaminated sediments on submerged vegetation: an experimental assessment of *Ruppia maritima*. PLoS One 10(10):e013879.

75) Atlas R.M.; Barsa R. (1992). Hydrocarbon biodegradation and oil spill bioremediation. Plenum Press, New York, p. 12.

76) Lahel A.; Fanta A.B.; Sergienko N.; Shakya M., Estefanía López M.; Behera S.K., Rene E.R., Park H.S. (2016) Effect of process parameters on the bioremediation of diesel contaminated soil by mixed microbial consortia. Int Biodeter Biodegr, 113: pp. 375–385.

77) Tumeo, M.; Braddock, J.; Venator, T.; Rog, S., Owens, D., (1994). Effectiveness of a biosurfactant in removing weathered crude oil from subsurface beach material. Spill Sci. Technol. Bull. 1: pp.53–59. http://dx.doi.org/10.1016/1353-2561(94)90007-8.

78) Pontes J.; Mucha A.P.; Santos H.; Reis I.; Bordalo A.; Basto M.C.; Bernabeu A.; Almeida C.M.R. (2013). Potential of bioremediation for buried oil removal in beaches after an oil spill. Mar Pollut Bull 76(1–2): pp. 258–265.

79) Xu J.; Kong F.; Song S.; Cao Q.; Huang T.; Cui Y. (2017) Effect of Fenton pre-oxidation on mobilization of nutrients and efficient subsequent bioremediation of crude oil-contaminated soil. Chemosphere 180: pp. 1–10.

80) Makadia TH; Adetutu EM; Simons KL; Jardine D; Sheppard PJ; Ball AS (2011). Re-use of remediated soils for the bioremediation of waste oil sludge. J. Environ. Manage. 92: 866-71.

81) Abdulsalam S.; Bugaje I. M.; Adefila S. S.; Ibrahim S. (2011). Comparison of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil. Int. J. Environ. Sci. Tech. 8 (1): pp. 187-194.

82) Andreoni V.; Gianfreda L. (2007). Bioremediation and monitoring of aromatic-polluted habitats. Appl. Microbiol. Biot. 76: pp. 287-308.

83) Gennaro P. D.; Morenoa B.; Annonib E.; García-Rodríguez S.; Bestetti G.; Beniteza E. (2009). Dynamic changes in bacterial community structure and in naphthalene dioxygenase expression in vermicompost-amended PAH-contaminated soils. J. Hazard. Mater. 172 (2-3): pp. 1464-1469.

84) Zahed MA, Aziz HA, Isa MH, Mohajeri L (2010). Effect of initial oil concentration and dispersant on crude oil biodegradation in contaminated seawater. B. Environ. Contam. Tox. 84 (4): pp. 438-442.

85) Sheppard PJ; Adetutu EM; Makadia TH; Ball AS (2011). Microbial community and ecotoxicity of bioremediated, weathered hydrocarbon-contaminated soil. Soil Research 49: pp. 261-269

86) Zabbey, N. ; Sam, K. ; Onyebuchi, A.T. (2017). Remediation of contaminated lands in the Niger Delta, Nigeria: Prospects and challenges. Science of Total Environment. http://dx.doi.org/10.1016/j.scitotenv.2017.02.075.

87) Environmental Protection Agency (EPA) (1997). Use of Monitored Natural Attenuation at Superfund, RCRA Corrective Action, and Underground Storage Tank Sites. Washington, D.C: Office of Solid Waste and Emergency Response.

88) Leal A.J.; Rodrigues E.M.; Leal P.L.; (2017). Changes in the microbial community during bioremediation of gasoline-contaminated soil. Braz J Microbiol; 48: pp. 342–51.

89) Declercq I.; Cappuyns V.; Duclos Y. (2012) Monitored natural attenuation (MNA) of contaminated soils: state of the art in Europe-a critical evaluation. Sci Total Environ; 426: pp. 393–405.

90) Megharaj M.; Ramakrishnan B.; Venkateswarlu K. (2011). (Bioremediation approaches for organic pollutants: a critical perspective. Environ Int 37: pp. 1362–75.

91) Roling W.F.M.; van Verseveld H.W. (2002). Natural attenuation: what does the subsurface have in store? Biodegradation, 13: pp. 53-64.

92) Dzionek A.; Wojcieszynska D.; Guzik U. (2016). Natural carriers in bioremediation: a review. Electron J Biotechn, 23: pp. 28–36.

93) Pepper I.L.; Gentry T.J.; Newby D.T. (2002). The role of cell bioaugmentation and gene bioaugmentation in the remediation of co-contaminated soils. Environ Health Persp, 110: pp. 943–6.

94) Mrozik A.; Miga S.; Piotrowska-Seget Z. (2011). Enhancement of phenol degradation by soil bioaugmentation with *Pseudomonas* sp. JS150. J Appl Microbiol, 111: pp. 1357–70.

95) Suja F.; Rahim F.; Taha M.R. (2014). Effects of local microbial bioaugmentation and biostimulation on the bioremediation of total petroleum hydrocarbons (TPH) in crude oil contaminated soil based on laboratory and field observations. Int Biodeter Biodegr , 90: pp. 115–22.

96) Shim H.; Hwang B.; Lee S.S. (2005). Kinetics of BTEX biodegradation by a coculture of *Pseudomonas putida* and Pseudomonas fluorescens under hypoxic conditions. Biodegradation 16: pp. 319–27.

97) Heinaru E.; Merimaa M.; Viggor S. (2005) Biodegradation efficiency of functionally important populations selected for bioaugmentation in phenol- and oil-polluted area. Fems Microbiol Ecol 51: pp. 363–73.

98) Sarkar D.; Ferguson M.; Datta R. (2005). Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation. Environ Poll. 136: pp. 187–95.

99) Gonzalez P.; Sanchez Y. (2011). Bioremediation of oil spills. Escuela Tecnica Superior de ingenieros de Minas.

100) Akaya FAQs"_Akaya. Retrieved 2015-09-14.

101) The Centre for Public Environmental Oversight (CPEO). Bioventing. Retrieved 2009-11-29, http://www.cpeo.org/techtree/ttdescript/bioven.htm.

102) Thomé, A.; Reginatto, C.; Cecchin, I.; Colla, L.M. (2014). Bioventing in a residual clayey soil contaminated with a blend of biodiesel and diesel oil. J. Environ. Eng. 140: pp. 1–6, http://dx.doi.org/10.1061/(ASCE)EE.1943-7870.0000863.

103) Bhakta, J. N. (2017). Handbook of Research on Inventive Bioremediation Techniques. IGI Global Publishers.

104) Margesin R.; Schinner F (2001). Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an alpine glacier skiing area. Appl. Environ. Microb. 67: pp. 3127-3133.

105) Johnsen A.R.; Wick L.Y.; Harms H. (2005). Principles of microbial PAH-degradation in soil. Environ. Pollut. 133: pp. 71-84.

106) Ellis, B. (1994). Reclaiming Contaminated Land: In situ/Ex situ Bioremediation of Creosote- and Petroleum Hydrocarbon- Contaminated Sites. Chapter 6. Bioremediation - Field experience by CRC Press, Inc.

107) Das N.; Chandran P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnol Res Int.; pp. 1-13.

108) Macaulay B.M. (2015). Understanding the behavior of oil-degrading micro-organisms to enhance the microbial remediation of spilled petroleum. Appl Ecol Environ Res.; 13(1): pp. 247-62.

109) Yang S.Z.; Jin H.J.; Wei Z. (2009). Bioremediation of oil spills in cold environments: A review. Pedosphere.; 19(3): pp. 371-81.

110) Vidali M.; (2001). Bioremediation; An overview, Pure Appl. Chem., 73(7): pp. 1163.

111) Montagnolli R.N.; Matos Lopes P.R.; Bidoia E.D. (2015). Assessing Bacillus subtilis biosurfactant effects on the biodegradation of petroleum products. Environ. Monit. Assess.; 187(4116): pp. 1-17.

112) Nasirpour N.; Mousavi S.M.; Shojaosadati S.A. (2015). Biodegradation potential of hydrocarbons in petroleum refinery effluents using a continuous anaerobic-aerobic hybrid system. Korean J. Chem Eng 32(5): pp. 874–881.

113) Rastegar S.O.; Mousavi S.M.; Shojaosadati S.A.; Sheibani S. (2011). Optimization of petroleum refinery effluent treatment in a UASB reactor using response surface methodology. J Hazard Mater 197: pp. 26–32.

114) Atlas R.M. (1991). Microbial hydrocarbon degradation—bioremediation of oil spills. J Chem Technol Biotechnol 52(2): pp. 149–156.

115) Enim A.E. (2013). Factors that Determine Bioremediation of Organic Compounds in the Soil. Academic Journal of Interdisciplinary Studies; 2(13): pp. 125-8.

116) Wang Q.; Zhang S.; Li Y. (2011). Potential Approaches to Improving Biodegradation of Hydrocarbons for Bioremediation of Crude Oil Pollution. Environ Protection J.; 2: pp. 47-55.

117) Cases I.; De Lorenzo V. (2005). Genetically modified organisms for the environment: stories of success and failure and what we have learned from them. International microbiology; 8: pp. 213-22.

118) Madhavi G.N.; Mohini D.D. (2012). Review paper on – Parameters affecting bioremediation. International journal of life science and pharma research; 2(3): pp. 77-80.

119) Ngene, S.; Tota-Maharaj K. (2019). Environmental technologies for remediation of contaminated lands in the Niger Delta region of Nigeria: Opport. for ecosystems services to host communities. UWE Bristol Research Repository: pp. 1-12.

120) Soleimani M.; Farhoudi M.; Christensen J.H. (2013) Chemometric assessment of enhanced bioremediation of oil contaminated soils. J Hazard Mater 254–255: pp. 372–381.

121) Baniasadi M.; Mousavi S.M.; Zilouei H.; Shojaosadati S.A.; lRastegar S.O. (2018). Statistical evaluation and process optimization of bioremediation of polycyclic aromatic hydrocarbon in a bioreactor. Environ Eng Manag J 17(8): pp. 1782–1790.

122) Sayler G.S.; Ripp S. (2000). Field applications of genetically engineered microorganisms for bioremediation processes. Curr Opin. Biotechnol. 11: pp. 286–289.

123) Kulshreshtha S. (2013). Genetically Engineered Microorganisms: A Problem Solving Approach for Bioremediation. J. Bioremed Biodeg. 4: e133. doi:10.4172/2155-6199.1000e133.

124) Chai L.; Jiang X.; Zhang F.; Zheng B.; Shu F.; Wang Z.; Cui Q.; Dong H.; Zhang Z.; Hou D.; She Y. (2015). Isolation and characterization of a crude oil degrading bacteria from formation water: comparative genomic analysis of environmental Ochrobactrum intermedium isolate versus clinical strains. J Zhejiang Uni SCI B 16(10): pp. 865–874.

125) Jafari M.; Rezaee D.Y.; Mohammadi G.E.; Varma A. (2013). Bioremediation and genetically modified organisms. In: Goltapeh EM, Danesh YR, Varma A (eds) Fungi as bioremediators; Springer, Berlin/Heidelberg, 32: pp 433–451.

126) Nezha T.J, Wifak B.; Hanane S.; Naïma E.G (2013). Biodegradation: Involved Microorganisms and Genetically Engineered Microorganisms. Biodegradation - Life of Science: pp. 290-320. http://dx.doi.org/10.5772/56194.

127) US Department of Energy https://www.energy.gov/sites/prod/files/2019 /11/f68/Products%20Made%20From%20Oil%20and%20Natural%20Gas%20Infographic.pdf

128) https://www.differencebetween.com/difference-between-aromatic-and-vs-aliphatic/

129) Omics in Aquatic Ecotoxicology Patrice Gonzalez, Fabien Pierron, in Aquatic Ecotoxicology, 2015

130) National Oceanic & Atmospheric Administration Website ……………………….. https://oceanservice.noaa.gov/facts /plankton.html, publication date: 02/26/21.

131) California Office of Health Hazard Assessment https://oehha .ca.gov/fish/mercury-fish-information-people-who-eat-fish

132) Centers for Disease Control and Prevention,……………………………………. https://www.cdc.gov/nceh/lead/prevention/health-effects.htm

133) Avogadro Constant for the Definition & Realization of the Mole First Published: 27 December 2018 Https ://www. Onlinelibrary .wiley.com Bernd Güttler, Olaf Rienitz, Axel Pramann, /doi/full/10.1002/andp.201800292

134) National Oceanic & Atmospheric Administration Website ……………………….. Office of Response & Restoration https://response.restoration.noaa.gov/oil-and-chemical-spills/oil-spills/oil-types.html

135) Royal Society Publishing ……………. A new definition for the mole based on the Avogadro constant: a journey from physics to chemistry. https://royalsocietypublishing.org/doi/10.1098/rsta.2011.0176 2.3

136) Amro, Mohammed & Benzagouta, Mohammed & Karnanda, Wimpy. (2011). Investigation on crude oil penetration depth into soils. Arabian Journal of Geosciences. 6. 10.1007/s12517-011-0392-6.

137) National Research Council 1993. In Situ Bioremediation: When Does it Work?. Washington, DC: The National Academies Press. https://doi.org/10.17226/2131.Pg 2

138) Sinha, A., Kumar, S. & Khare, S.K. Biochemical Basis of Mercury Remediation and Bioaccumulation by *Enterobacter* sp. EMB21. *Appl Biochem Biotechnol* **169,** 256–267 (2013). https://doi.org/10.1007/s12010-012-9970-7