# BIOREMEDIATION OF ARTIFICIALLY LUBRICANT CONTAMINATED SOIL WITH MANURE

**A Dissertation**

Submitted in partial fulfillment of the requirement for the award of degree of

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in

**ENVIRONMENTAL SCIENCE AND TECHNOLOGY**

By:

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## CERTIFICATE

This is to certify that thesis entitled "**Bioremediation of Artificially Lubricant Contaminated Soil with Manure**" submitted by Mr. Sandeep Singh in partial fulfilment of the requirements for the award of Master of Technology in **Environmental Science and Technology** at Thapar University, Patiala (Deemed University) is an authentic work carried out by him under our supervision and guidance.

To the best of our knowledge, the matter embodied in this thesis has not been submitted to any other university/institute for award of any degree/ diploma.

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## DECLARATION

I, the undersigned, hereby declare that the research work presented in the M.Tech project entitled "**Bioremediation of Artificially Lubricant Contaminated Soil with Manure**" has been carried out by me under the supervision of Dr. Anita Rajor, Associate Professor, School of Energy and Environment, Thapar University, Patiala.

Further, I declare that no part of this Dissertation has been submitted for a degree or any other qualification of any other university or examining body in India/elsewhere.

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# BIOREMEDIATION OF ARTIFICIALLY LUBRICANT CONTAMINATED SOIL WITH MANURE


## ABSTRACT

Petroleum products are the richest source of energy in the present World. Oil spills during automobile refilling are one of the most common types of soil pollution in the vicinity of oil refilling stations and the main problem is the degradation of such petroleum products. Bioremediation has become an attractive alternative to physicochemical methods of remediation, where feasible.

The use of agriculture waste in the form of manure is gaining much importance over microbial isolates in the treatment of oil contaminated sites. The purpose of the use of compost is that it is easy available, containing consortia of microorganisms, increases soil fertility and above all minimization of agriculture residues. In the present study compost was utilized for the treatment of oil contaminated soil. Lubricant oil (5000ppm) was added to sterile soil to form synthetic sample and compost was added in diff. proportions ranging from 0-75%. Early investigation revealed increase in total microbial count and decrease in total petroleum hydrocarbon. Further study will be focused on the physical, chemical and biological parameters of soil containing manure.



**Keywords**: Manure, Lubricant Oil, Soil, Total Petroleum Hydrocarbon

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# CHAPTER 1

# INTRODUCTION

## 1.1 General

Petroleum is one of the rich source of energy and organic matter for today's world. Oil refineries are facing problems for disposal of oil sludge or automobile oil. Generally bioremediation is a good method for this type of treatment. The maximum work has been done on soil with lower concentration of pollutants. Contaminated soils generally have low organic matter with limited microbial activity. The bacteria present in manure degrade the contaminant present in the soil. However, it is important to know the ratio of contaminated soil to organic amendments it is necessary because an inappropriate amount may retard or inhibit microbial activity. The treated soil sample will be characterized for physical, chemical and biological parameters. Despite its important usage, petroleum hydrocarbons also pose as a globally environmental pollution. The whole world, especially the petroleum oil producing countries, are vulnerable to oil spills due to the large volume of petroleum oil and its products transported from producing end to consumer end.

Petroleum hydrocarbons are one of the most frequently encountered pollutants in soil habitats due to the increased usage of petroleum products and the seemingly increasing probability of accidents (Samanta et al. 2002). The most noticeable sources of contamination are releases from manufacturing and refining installations, oil-tanker spills and accidents during transportation of the oil. Crude oils are transported long distance either on land pipeline or on water in tankers and both of them are prone to oil spill and accidents. These Pollutants interact with soil microorganisms upon their entry to the soil ecosystem. After the normal metabolic activities and functions of soil microorganisms are hindered by pollution, microbial nutrient processes and enzyme activities as well as soil fertility and production will be affected or even collapse (Topac 2011). Remediation of the contaminated soil can be done in many ways which include thermal desorption, thermal destruction (or Incineration), soil washing and flushing, chemical treatment, biological remediation, vacuum extraction, chemical extraction and solidification. Biological methods can be cheaper, easier and ecofriendly than efficient physical and chemical methods**.**

Bioremediation is a process by which chemical substances are degraded by microorganisms that certain microorganisms are able to degrade petroleum hydrocarbons and use them as a sole source of carbon and energy for growth. Bioremediation is not a new idea. Microbiologists have studied the process since the 1940s. However, bioremediation became recognized to a broader public in the U.S. only in the late 1980s as a technology for cleanup of shorelines contaminated with spilled oil.

Bioremediation has become an attractive alternative to physicochemical methods of remediation. Manure have been shown to stimulate the degration of petroleum hydrocarbons in soil, and it was hypothesized that the role of manure in remediation lies in the enhancement of an oil degrading microbial community.

Microorganism are the major agents in the degradation of petroleum hydrocarbons. The organisms include bacteria, yeast, filamentous fungi and algae. The principal bacteria and fungi generally responsible for oil degradation in both soil and aquatic environment have been identified as comprising mainly *pseudomonas, Achro-bateri , Bacillus, Micrococcus, Nocardia, Trichoderma, Penicillium, Aspergilllus* and *Morteilla.*(Ezeji et al. 2005)

## 1.2 Lubricant Oil

A **lubricant oil** is a substance introduced to reduce friction between moving surfaces. It may also have the function of transporting foreign particles. The property of reducing friction is known as lubricity (Slipperiness).One of the single largest applications for lubricants, in the form of motor oil, is protecting the internal combustion engines in motor vehicles and powered equipment. Typically lubricants contain 90% base oil (most often petroleum fractions, called mineral oils) and less than 10% additives. Vegetable oils or synthetic liquids such as hydrogenated polyolefins, esters, silicones, fluorocarbons and many others are sometimes used as base oils. Additives deliver reduced friction and wear, increased viscosity, improved viscosity index, resistance to corrosion and oxidation, aging or contamination, etc.

Lubricants such as 2-cycle (2T) oil are added to fuels like gasoline which has low lubricity. Sulfur impurities in fuels also provide some lubrication properties, which has to be taken in account when switching to a low-sulfur diesel; biodiesel is a popular diesel fuel additive providing additional lubricity.

Non-liquid lubricants include grease, powders (dry graphite, PTFE, molybdenum disulfide, tungsten disulfide, etc.), PTFE tape used in plumbing, air cushion and others. Dry lubricants such as graphite, molybdenum disulfide and tungsten disulfide also offer lubrication at temperatures (up to 350 °C) higher than liquid and oil-based lubricants are able to operate. Limited interest has been shown in low friction properties of compacted oxide glaze layers formed at several hundred degrees Celsius in metallic sliding systems, however, practical use is still many years away due to their physically unstable nature.

Another approach to reducing friction and wear is to use bearings such as ball bearings, roller bearings or air bearings, which in turn require internal lubrication themselves, or to use sound, in the case of acoustic lubrication. For the good performance of the lubricant oil some additives are used to import some specific properties to the finished oil. Additives for lubricating oils were used first during the 1920s, and their use has since increased tremendously. Today, practically all types of lubricating oil contain at least one additive, and some oils contain additives of several different types. The amount of additive used varies from a few hundredths of a percent to 30% or more. Over a period of many years, oil additives were identified that solved a variety of engine problems: corrosion inhibition, ability to keep particles such as soot dispersed, ability to prohibit acidic combustion products from plating out as varnish on engine surfaces, and ability to minimize wear by laying down a chemical film on heavily loaded surfaces. In addition, engine oil became specialized so that requirements for diesel engine oils began to diverge from requirements for gasoline engines, since enhanced dispersive capability was needed to keep soot from clumping in the oil of diesel engines.

### 1.3 Impact Of Lubricant Oil On Environment and Health

The maximum focus was on the properties of used lubricant oil because most of the mineral-based lubricant oil at hazardous waste sites is expected to be previously used oil. It is important to make the distinction between new and used lubricant oil because the characteristics of lubricant oil change with use (Vazquez-Duhalt 1989). An important difference between new and used motor oil is the heavy metal content. This difference is extremely important because many of the metals are harmful to human health and living organisms. These metals originate from the fuel and from motor wear. Used oil contains high concentrations of lead, zinc, calcium, barium, and magnesium along with lower concentrations of iron, sodium, copper, aluminium, chromium, manganese, potassium, nickel, tin, silicon, boron, and molybdenum (Vasquez-Duhalt 1989). Concentrations of lead in used lubricant oil were likely higher when leaded gasoline was used.

Lubricant oils are manufactured using highly refined base oils and contain up to 20% of a variety of additives such as viscosity index improvers, detergents/dispersants, antiwear additives, pour-point depressants, and antioxidants (IARC 1984; Kirk-Othmer 1981). During use, the high temperatures and friction cause changes such as oxidation, nitration, and cracking of polymers in the component chemicals (Vazquez-Duhalt 1989). In addition, a variety of substances such as fuel, water, antifreeze, dust, and various combustion products such as polycyclic aromatic hydrocarbons (PAHs), metals, and metallic oxides accumulate in the oil. The degree of chemical change and accumulation of contaminants in the oil increases with use and varies depending on the type of fuel used and the mechanical properties of the engine.

Several of the oil additives are toxic environmental contaminants, e.g., zinc dithiophosphate and zinc diary1 or dialkyl dithiophosphates (ZDTPs); calcium alkyl phenates; magnesium, sodium, and calcium sulphonates; tricresyl phosphates; molybdenum disulfide; heavy metal soaps; and other organometallic compounds that contain heavy metals. Hence, very high levels of zinc and cadmium are found in new motor oil-approximately 1,500 μg/g of zinc and 87 μg/kg of cadmium (Hewstone 1994; Vasquez-Duhalt 1989). Although ZDTPs have a low acute systemic toxicity, they can cause eye damage and skin irritation (Hewstone 1994). Prolonged exposure to high concentrations of ZDTPs, calcium alkyl phenates, and magnesium, sodium, and calcium sulphonates had significant effects on the reproductive organs of male rabbits (testicular atrophy and reduction or absence of spermatozoa) which appeared to be species specific. The absorption of tricresyl phosphates caused peripheral nervous system damage, leading to neuromuscular problems (Hewstone 1994).

In a crankcase-lubricated engine, the oil compartment acts as a sink for heavy molecular incomplete combustion products such as PAHs and their analogs (Scheepers et al. 2001). Thus, contaminants such as PAHs, which are formed via combustion, can accumulate in the oil by a factor of up to 1,000. PAHs are known to be highly toxic environmental contaminants with carcinogenic and mutagenic properties. They leave the engine in various ways, such as via particulates, oil leaks, and uncontrolled oil changes, which then accumulates in the environment. One hundred and forty different PAHs have been found in the used oil of crankcase-lubricated engines. These PAHs are also preseni'in much lower quantities in new or fresh oil (Van Donkelaar 1990).

Used lubricant oil is a complex mixture of metals and PAHs. When motor oils undergo thermal decomposition, gasoline combustion products are formed, significantly increasing the levels of PAHs which contribute to the carcinogenic and mutagenic properties of the oils (Bingham 1988; Ingram et al. 1994). Hence, it is difficult to define the precise composition of used mineralbased crankcase oil because of the variety of chemical additives that may be present and the varying degrees of chemical decomposition and contaminant accumulation. Therefore, rather than describing toxicities associated with individual components, the following discussion focuses on information obtained in studies that have examined the effects of exposure to samples of used lubricant oil. In several studies, composite samples of used mineral-based crankcase oil have been employed; these studies may therefore provide a more generalized picture of toxicities associated with exposure. However, it should be noted that the results of any one study may not be representative of effects occurring with similar exposures to other samples of used lubricant oil.

### 1.3.1 Used Lubricant oil and environmental damage

Oily sluge is carcinogenic and a potent immunotoxicant (Propst et al. 1999). Improper disposal and handling of oily sludge contaminates soil and may pose a serious threat to groundwater. Once lubricating oil is drained from engines, gearboxes, hydraulic systems, turbines and air compressors: the oil is contaminated with wear debris, the lubricating base oil has deteriorated and degraded to acids, the additives have decomposed into other chemical species, process fluids, degreasers and solvents have mixed into the used oil.

Used oil contains wear metals such as iron, tin and copper as well as lead from leaded petrol used by motorists. Zinc arises from the additive packages in lube oils. Many organic molecules arise from the breakdown of additives and base oils. The molecule potentially the most harmful is the polycyclic aromatic hydrocarbon (PAH) such as benz(a)pyrene and chrysene. Petrol engines generate the most PAH molecules per 1000km, with diesel engines below that and 2 stroke engines generating the least amount of PAH. A release of used oil to the environment, whether by accident or otherwise, threatens ground and surface waters with oil contamination there by endangering drinking water supply and aquatic organisms. Bioremediation offers a promising means to reclaim such contaminated soil (Bartha 1986; Bragg et al 1994)

Used oil can damage the environment in several different ways:

- Spilled oil decomposes very slowly and tends to accumulate in the environment, causing soil and water pollution. It reduces the oxygen supply to the micro-organisms that break the oil down into non-hazardous compounds.
- Toxic gases and harmful metallic dust particles are (metal ions, lead, zinc, chromium and copper) produced by the ordinary combustion of used oil and can be toxic to ecological systems and to human health if they are emitted from the exhaust stack of uncontrolled burners and furnaces.
- Some of the additives used in lubricants can contaminate the environment (e.g.zinc dialkyl dithiophosphates, molybdenum disulphide, and other organo-metallic compounds.)

- Certain compounds in used oil such as poly-aromatic hydrocarbons (PAH) can be very dangerous to health due to carcinogenic and mutagenic. The PAH content of engine oil increases with operating time, because the PAH formed during combustion in petrol engines accumulates in the oil.
- Lubricating oil is transformed by the high temperatures and stress of an engine's operation resulted in oxidation, nitration, cracking of polymers and decomposition of organ- metallic compounds.
- Other contaminants also accumulate in oil during use of fuel, antifreeze/coolant, water, wear metals, metal oxides and combustion products.

As it is already mentioned above that if used oil is disposed in irresponsible manner it may cause great danger to the human resources like drinking water. The environmental effects of used oil can be categorized as follows.

1. On human health
2. Wetlands and wildlife
3. Burning waste oil
4. Marine and fresh water organisms
5. Using waste oil as dust control agent

Thousands of tons of oil are discharged into nature causing important environmental problems and leading to the need of alternative lubricant products with minimal environmental impact. Oily soil and sludges is carcinogenic and a potent immunotoxicant (Propst et al. 1999). Even improper disposal and handling of oily sludge contaminates. Soil and lubricant may pose a serious threat to ground water. The environmentally ecofriendly method to remediate such contamination soil is the bioremediation.( Bragg et al. 1994). Thus, a major environmental impact contribution in the biodegradable lubricant process comes from the bioremediation.

From the above observations following objectives were framed:

1. Determination of total hydrocarbon content in automobile oil contaminated soil.
2. Degradation studies of automobile oil by using different concentrations of organic manure.

# CHAPTER 2

## REVIEW OF LITRATURE

Petroleum is a rich source of energy but crude oil pollution adversely effects the soil and water system. The adsorption of oil in soil particles provides excess of carbon which might be unavailable for microbial use, which create limitation in soil nitrogen and phosphorous (Atlas 1981, Baner and Herison 1994). According to Plohl et al 2002, petroleum continuously used as the principal source of energy, however, despite its important usage, petroleum hydrocarbons also pose as a globally environmental pollutant. The whole world, especially the petroleum oil producing countries, are vulnerable to oil spills due to the large volume of petroleum oil and its products transported from producing end to consumer end. For example, in the Niger Delta of Nigeria alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing about 2.8 million barrels of crude oil into the environment (Korie-Siakpere 1998, Odiete 1999). According to Obayori et al 2008, petroleum product contamination has been further compounded by sabotage and vandalization of pipelines in restive communities, particularly in the Niger Delta region of Nigeria. In 2010, the oil leak by the British oil giant, British Petroleum, in the Gulf of Mexico in the United States has been described by the environmental experts as the worst in history.

According to Nwaogu et al. (2008), diesel oil is one of the major products of crude oil that constitutes the main source of pollution in the environment. With the increasing demand for diesel oil in cars, trucks, generators and industrial machines, combined with large quantities of the oil being transported over long distances, the contamination of soils through accidental spillage, cleaning of machines and tankers could reach an alarming state. Petroleum products are considered to be recalcitrant to microbial degradation and persist in ecosystems because of their hydrophobic nature and low volatility and thus they pose a significant threat to the environment (Abed et al. 2002). According to Boonchan et al. (2000) and Samanta et al. (2002) the constituents of petroleum such as diesel oil, are carcinogenic, mutagenic and are potent immunotoxicants, thus posing a serious threat to human and animal health. It has been established that, the biodegradation of hydrocarbon-contaminated soils, by exploiting the ability of microbes to degrade and/or detoxify organic contaminants, is an efficient, economic, versatile and environmentally sound treatment (Mehrashi et al. 2003). Hence, ability of microbes to degrade organic contaminants has formed the basis for bioremediation in the field. However, a number of factors must be considered before *in situ* bioremediation can be effectively applied. These factors include (i) type and concentration of oil contaminant, (ii) prevalent climatic conditions, (iii) type of environment that has been contaminated, and (iv) nutrient content and (v) pH of the contaminated site (Rosengerg 1992).

Degradative strains of mycobacterium spp. are commonly isolated from polycyclic aromatic hydrocarbon (PAH) contaminated soil. Mineralization results obtained from a sterilized soil inoculated with strain RJGII-135 suggested that competition with indigenous microorganisms may be a significant factor affecting bio degradation of PAHs. Pyrene-amended soils, with and without inoculation with strain RJGII-135, experienced both increases and decreases in the population sizes of the inoculated strain and indigenous mycobacterium population during incubation (Cheung and Kinkle 2001). Polycyclic aromatic hydrocarbon (PAHs) consist of a class of chemicals with two or more fused benzene rings in linear, angular, or cluster arrangements. PAHs are ubiquitous, they are prodused during fossil fuel combustion, waste incineration, or as by-products of industrial process, such as coal gasification and petroleum refining, and are often released in large quantities into the environment. High molecular weight PAHs are important constituents of petroleum as they are recalcitrant pollutants and because several of them are known mutagens or carcinogens for example, the four-ring pyrene is mutagenic, whereas the five ring benzo pyrene is both mutagenic and carcinogenic (Cerniglia 1992).

Nwaogu et al. (2008) uses *Bacillus subtilis* for the degradation of diesel oil in a polluted soil. they use six month oil diesel oil for source for isolation of *Bacillus subtilis*, *Bacillus cereus*, *Trichoderma hazanium* and *Trichothercium reseum*. These organism were found to be hydrocarbon degraders. Further it was found that B.Subtilis had higher potential to utilize diesel oil as carbon source. The contaminated soil sample with diesel oil 5% (v/w) and inoculated with *B.Subtilis* isolates. The degradation of the diesel oil was monitored over a twenty seven day period, using gravimetric method. The rates of degradation of diesel oil by the isolate at the end of 1, 12, and 27 day were $5.8\times10^{-4}$, $1.83\times10^{-3}$ and $1.05\times10^{-3}$ g/h respectively. Agamuthu et al. (2013) used different selected organic waste for the remediation of hydrocarbons in contaminated soil. they use sewage sludge and cow dung and found that cow dung amended set ups showed 94% biodegradation while sewage sludge amendment gave 82% degradation after 98 days and 10% w/w amends. As for the microbial counts, cow dung amended soil recorded 69-122×$10^7$ cfu/g while sewage sludge amended soil recorded 63-96×$10^7$ cfu/g.

Facundoj et al (2000) studied the biodegradation of diesel oil in soil by microbial consortium. They observed the concentration of diesel diesel in soil treated with the bacterial consortium was reduced to <15% of the initial concentration, with in a period of five weeks in laboratory and pilot scale. The enhancement of the microbial activity in hydrocarbon contaminated soil can be achieved with the combination of stepwise soil inoculation and nutrient addition. Abioye et al (2012) studied the bioremediation of soil contaminated with 5% and 15% (w/w) used lubricant oil and amended it with 10% brewery spent grain (BSG), banana skin (BS), and spent mushroom compost (SMC) and observed it for 84 days, under laboratory conditions. After 84 days the highest percentage of oil biodegradation (92%) was recorded in soil contaminated with 5% used lubricating oil and amended with BSG, while only 55% of oil biodegration was recorded in soil contaminated with 15% used lubricating oil amended with BSG. Abioye and Aziz (2009) used banana skin (BS), brenery spent grain (BSG) and spent mushroom compost (SMC) for bioremediation of soil contaminated with used lubricant oil. These organic wastes enhancing biodegradation of the oil were studied for a period of 84 days under laboratory condition. Hydrocarbon utilizing bacterial count were high in all the organic waste amended soil rangind between 10.2×$10^6$ and 80.5×$10^6$ cfu/g compared to control soil throughout the 84 days of study. Oil contaminated soil amended with BSG showed the highest reduction 26.76% of oil content in 84 days compare to other treatments. The results demonstrated the potential of organic waste for oil bioremediation in the order BSG>BS>SMC. Dadrasnia and Agamuthu (2013) used three different organic wastes tea leaf, soy cake and potato skin for a period of 3 months for the degradation of oil in oil contaminated soil. They observed 35% oil loss within 84 days in control soil while 88, 81 and 75% oil degradation recorded in the soil amended with soy cake, potato skin and tea leaf respectively. The diesel fuel utilizing bacteria isolated from the oil contaminated soil belongs to *Bacillus licheniformis*, *Ochrobactrum tritici* and *Staphylococcus sp*. Soil amended with soy cake recorded the highest oil biodegradation (53%) compared to other treatments. Hadibarata and Tachibana (2009) use white rot fungus *Polyporus sp*. S133 collected from petroleum contaminated soil was tested for its ability to grow and degrade crude oil, obtained from petroleum industry. The ability of *Polyporos sp.* S133 pre grown on wood meal to degrade crude oil was measured. Maximum degradation (93%) was obtained when polyporus sp. S 133 was incubated in 1000 mg/L of crude oil for 60 days, as compared to 19% degradation rate in 15000 mg/L. Increased concentration of crude oil decreased the degradation rate. Vilches et al 2010 uses whey and nutrient for the enhanced natural biodegradation of diesel fuel contaminants in soil. in the field study, biodegradations profiles were monitored under controlled laboratory conditions by taking soil samples from contaminated site. The experiments were carried out by adding whey and mineral nutrients (NPK) to the test area and monitor the degradation of hydrocarbons by gas chromatographic analysis of extracted soil samples. Significant effects on the degradation rates were achieved.

In 2007, crude oil was discovered in Ghana at Cape Three Point in the Western Region. In the closing days of 2010 when the country joined the league of oil producing countries and when the Jubilee Oil Field officially came on stream with the pumping of oil in commercial quantities, the country had started experiencing oil spillage. On December 26, 2009, the country experienced its first spillage of about 584 barrels of low-based mud drilling fluid and the second mud spill of seven barrels occurred on March 23, 2010 (Ghanaian Daily Graphic 2010). This frequency of spillage in the drilling field of Cape Three Point is of great concern. Little information is known about the degradation potential of Ghanaian soils regarding petroleum product(s) contamination. The present study therefore investigated the degradation of diesel oil in four soils obtained from different agro-ecological zones in Ghana with the aim to assess the diesel oil degrading potential of these soils as a preliminary research finding in the country to assist in bioremediation of soils contaminated with petroleum product(s) in the future. Some studies has also been done in which vegetable waste and saw dust were added to the contaminated soil as the nutrient and bulking agent respectively in the ratio of 0.6:1 and 0.5:1. Neutral pH and 50% moisture content were maintained in both the reactors throughout the experiment. Oil content of the bioremediated soil was measured every 2 days during the period of study. Results indicated that the supplement of amendments significantly increased the indigenous microbial population in soil and thus enhanced the oil degradation. Maximum remediation occurred in the reactor with the amendment where the oil removal efficiency was found to be 74%.

Zhang et al. (2010) found a collection of 38 bacteria by enrichment cultivation from oil contaminated soil of an oil field in Daqing, China. Twenty two strains could utilize diesel oil as the sole source of carbon and energy and 11 strains could degrade the TPHs of diesel oil by more than 70% in 7 days. Nineteen of the bacteria were related to *Bacillus* species. About 87.5% TPHs of cruid oil were degraded by a consortium of seven strains. The collection of isolated bacteria might be a useful resource for bioremediation of oil-contaminated soil.

According to Gopamma and Srinivas (2011) the use of organic manure or compost in bioremediation of soil contaminated with lubricant oil.

The treatment of soil samples were characterized for pH, microbial respiration, carbon and heterotrophic bacterial count. The experiment results showed enhanced biodegradation of lubricant oil due to organic manure addition. The maximum removal was 19.8% after four week study period at 75% of amendment. The result showed that nutrient supplementation enhanced the biodegradation rate of lubricant oil.

## 2.1 Disposal and Environment

It is estimated that 40% of all lubricants are released into the environment. Recycling, burning, landfill and discharge into water may achieve disposal of used lubricant. There are typically strict regulations in most countries regarding disposal in landfill and discharge into water as even small amount of lubricant can contaminate a large amount of water. Most regulations permit a threshold level of lubricant that may be present in waste streams and companies spend hundreds of millions of dollars annually in treating their waste waters to get to acceptable levels.

Burning the lubricant as fuel, typically to generate electricity, is also governed by regulations mainly on account of the relatively high level of additives present. Burning generates both airborne pollutants and ash rich in toxic materials, mainly heavy metal compounds. Thus lubricant burning takes place in specialized facilities that have incorporated special scrubbers to remove airborne pollutants and have access to landfill sites with permits to handle the toxic ash.

Unfortunately, most lubricant that ends up directly in the environment is due to general public discharging it onto the ground, into drains and directly into landfills as trash. Other direct contamination sources include runoff from roadways, accidental spillages, natural or man-made disasters and pipeline leakages.

Improvement in filtration technologies and processes has now made recycling a viable option (with rising price of base stock and crude oil). Typically various filtration systems remove particulates, additives and oxidation products and recover the base oil. The oil may get refined during the process. This base oil is then treated much the same as virgin base oil however there is considerable reluctance to use recycled oils as they are generally considered inferior. Basestock fractionally vacuum distilled from used lubricants has superior properties to all natural oils, but cost effectiveness depends on many factors. Used lubricant may also be used as refinery feedstock to become part of crude oil. Again there is considerable reluctance to this use as the additives, soot and wear metals will seriously poison/deactivate the critical catalysts in the process. Cost prohibits carrying out both filtration (soot, additives removal) and re-refining (distilling, isomerisation, hydrocrack, etc.) however the primary hindrance to recycling still remains the collection of fluids as refineries need continuous supply in amounts measured in cisterns, rail tanks. Lubricants both fresh and used can cause considerable damage to the environment mainly due to their high potential of serious water pollution and soil pollution. Further the additives typically contained in lubricant can be toxic to flora and fauna. In used fluids the oxidation products can be toxic as well. Lubricant persistence in the environment largely depends upon the base fluid, however if very toxic additives are used they may negatively affect the persistence.

## 2.2 EFFECTS OF HYDROCARBONS

### 2.2.1 *On Plant*

Kisic et al. (2007) studied a four-year pot trial was set up to determine, as precisely as possible, the influence of increased levels of TPH upon soil and plants grown. In eight treatments, clean soil and different doses of drilling fluids and crude oil were applied. The changes in some chemical parameters of soil, plant density and crop yields were investigated. The influence of the studied indicators on the achieved plant density and crop yield was strongest in the first trial year. Drilling fluids had a stronger impact on the chemical properties of the studied soil, while plant density and yield were more strongly affected by crude oil. Upon application of drilling fluids and crude oil, the soil pH, contents of organic matter and heavy metals varied very little throughout the trial period, whereas the soil levels of total petroleum hydrocarbons, mineral oils and polycyclic aromatic hydrocarbons were significantly reduced after the first trial year. Peng et al. (2009) has been done on phytoremediation by using Jatropha curcas plant observed that hydrocarbons was not accumulated by jatropha roots from the soil but the microbes which are present to the plant root and they are the one who degrade the hydrocarbon, so it was concluded that degradation is via rhizo. Several researchers use non edible plants for phyto remediation. Alfalfa and Horse radish are also commonely used plant which was found to reduce the contamination. some plants for successfully removal of petroleum hydrocarbon. A certain amount of TPH could serve as fertilizer and stimulate plant and animal growth (Kisic et al. 2009). The reason for reduced plant growth in soil contaminated by TPH range from direct toxic effects of oil on plants ( Bake 1970, Kyung-Hwa et al 2004). Lack of germination due to the lack of viable seeds (Kudayo et al. 2001; Ogboghodo et al. 2004), reduced germination (Dorn and Salanitro 2000) and unsatisfactory soil conditions.

2.2.2 *Soil Temperature and Moisture Regimes*

Comparisons between temperature profiles of hydrocarbon contaminated sites indicate that during sunny weather, when soils are snow-free, the daily maximum surface temperature of hydrocarbon-contaminated soils is often warmer (by up to 10 °C) than adjacent sites. (Balks et al. 2002). The higher temperatures at the hydrocarbon-contaminated sites were attributed to decreased soil surface albedo due to surface darkening by hydrocarbons. In contrast, site where hydrocarbons contaminated the subsurface, no difference in soil temperature was detected in hydrocarbon-contaminated soils. There is potential for hydrocarbons to affect soil moisture regimes. Hydrocarbon-contaminated soils were weakly hydrophobic, whereas no evidence of hydrophobicity was detected at other sites. However, the small increase in hydrophobicity was considered unlikely to alter moisture penetration into the soil. No differences in moisture retention were found between hydrocarbon-contaminated and normal soil (Balks et al. 2002).

2.2.3 *Soil Chemical Properties*

Some soil chemical properties may be impacted by hydrocarbon spills . In particular, hydrocarbon spills on mineral soils can lead to a substantial increase in soil carbon. While the carbon content of normal soils was low (0.02 and 0.12%), following contamination with hydrocarbons the soil carbon content exceeded 5%. Levels of nitrate in hydrocarbon-contaminated soils were depleted compared with normal soils, a possible consequence of microbial activity in the contaminated soils. Total phosphorous levels, however, appeared unaffected by hydrocarbon contamination. The bulk soil pH values of the surface hydrocarbon-contaminated soils were lower than the corresponding normal sites, possibly indicating the accumulation of acidic microbial metabolites derived from hydrocarbons (Vincent et al 2011).

The biodegradability of certain biofuels was studied in the case of forest soils using themanometric respirometric technique, which was proved to be very suitable for untreated, fertilized as well as pH adjusted soils. Kaakineen et al. (2007) studied biological oxygen demand (BOD)/ theoretical oxygen demand (ThOD) of forest soil value of 45.1% was observed for a typical model substance, that is, sodium benzoate after a period of 30 days. The BOD/ThOD value is increased 76.2% by addition of nutrients did not biodegrade at all in 30 days in nonprocessed soil, and when pH was adjusted to 8.0. The BOD/ThOD value increased slightly to a value of 7.4% after adjustment of pH 8.0. Mineral addition improved the BOD/ThOD value on average to 43.2% after 30 days. The combined mineral addition and pH adjustment together increased the BOD/ThOD value to 75.8% in 30 days. The observations were similar with a rapeseed oil-based lubricating oil: after 30 days, the BOD/ThOD value increased from 5.9% to an average value of 51.9%, when the pH and mineral concentrations of the soil were optimized. The mineral addition and pH adjustment also improved the precision of the measurements significantly (Juhani et al. 2007). They observed crude oil pollution caused a reduction in pH, conductivity and phosphorous. The C/N ratio was increase compaired to animal waste and oil contain varying proportions of nitrogenous compounds and high amount of carbon.

### 2.2.4 *Soil Biota*

Contamination of soil used by lubricating oil is rapidly increasing due to population increase. The use of different types of automobiles and machinery has increased the use of lubricating oil. The spillage of all types of oil from different use contaminates environment with hydrocarbon. Petroleum hydrocarbons released into the environment can create risk to eco system and too human health. As the usage of petroleum hydrocarbon products increases, soil contamination with diesel and engine oil is becoming one of the major environmental problems. Mandari and Lin (2007) suggested effective and efficient strategy to speed up the clean up process. The addition of inorganic or organic nitrogen rich nutrients is an effective approach to enhance the bioremediation process (Semple et al. 2006; Walworth et al. 2007). The prevailing low temperatures, low humidity, freeze-thaw cycles, and salinity of Antarctic soils combine to create a harsh environment for soil biota. Few plants and animals have managed to colonize and survive in the terrestrial environment. Investigations of the effects of hydrocarbons on Antarctic soil biota have therefore focused on microbes. more recent investigations derive from interest in the potential application of bioremediation for cleanup of hydrocarbon-contaminated Antarctic soils. Spillage of hydrocarbons on Antarctic soils can result in enrichment of hydrocarbon degrading microbes so that they become a significant proportion of the total culturable microbiota. Numbers of hydrocarbon degraders are often low or below detection limits in normal soils (although ornithogenic soils may be an exception), whereas $>10^5$ hydrocarbon degraders $g^{-1}$ have been cultivated from contaminated soils . In contrast, culturable heterotrophic bacteria were only 1-2 orders of magnitude higher in hydrocarbon-contaminated than normal soils . Culturable yeasts were not detected in normal soils, yet reached $>10^5$ colony forming units $g^{-1}$ dry wt. in contaminated soils. These results indicate that hydrocarbon contaminants in Antarctic soils can serve as substrates for microbial growth and result in an enhanced number of culturable bacteria and increased proportions of hydrocarbon-degrading bacteria. Organic lead levels at the site were 25-fold higher than those detected in soils from normal sites. Significant rates of mineralization of radiolabeled dodecane and naphthalene have been measured in microcosms containing contaminated soils but not in nearby normal soils. This indicates that hydrocarbon degraders can be active in the soils under conditions similar to those in situ. The observed persistence of hydrocarbons in soils over decades however, indicates that in situ biodegradation rates must be very slow. Hydrocarbon-degrading bacteria isolated from contaminated Antarctic soils have been assigned to a number of genera including *Rhodococcus*, *Acinetobacter*, *Pseudomonas*, and *Sphingomonas*. All the hydrocarbon-degrading bacteria reported thus far are psychrotolerant rather than psychrophilic. while they could grow at low temperatures, their optimum temperature for growth was greater than 15 °C. Given the temperatures that surface soils can reach in summer ( 20 °C), the paucity of psychrophiles restricted to low temperatures is not surprising.

It was observed one of the isolates, *Pseudomonas stutzeri* 5A, grew on the aromatic compounds toluene, benzene, and *m*-xylene, whereas *Pseudomonas* sp. 5B utilized hexane and dodecane vapors. Their results suggest that such bacteria may contribute both to hydrocarbon degradation and nutrient cycling in situ. Whereas the presence of hydrocarbons in Antarctic soils can result in increased abundance of culturable microbes, a concomitant decrease is typically observed in overall microbial community diversity. At drilling sites where diesel fuel has been spilled, ammonifiers and hydrocarbon-degrading microbes may become predominant. Furthermore, shifts in the predominant fungal species of soils have been noted. Specifically, *Phialophora* spp. were more abundant in hydrocarbon-contaminated soils, whereas *Geotrichum* and *Chrysosporium* dominated pristine soils, and yeasts were isolated only from contaminated soil . To determine the impacts of hydrocarbon contamination on the diversity of bacterial communities in Ross Sea region coastal soils, a culture-independent phylogenetic survey and traditional culturing methods were combined . Soil samples were taken from two soil depths at a hydrocarbon contaminated site and normal sites. Small subunit rRNA genes were amplified directly from extracted soil DNA or from purified bacterial isolates using universal *Bacteria*-specific primers. Proteobacteria, specifically members of the genera *Pseudomonas*, *Sphingomonas*, and *Variovorax*, dominated the contaminated soils. (Jackiem et al. 2007)

## 2.3 Manure

Manure is organic matter used as organic fertilizer in agriculture. Manures contribute to the fertility of the soil by adding organic matter and nutrients, such as nitrogen, that are trapped by bacteria in the soil. Higher organisms then feed on the fungi and bacteria in a chain of life that comprises the soil food web. It is also a product obtained after decomposition of organic matter like cow-dung which replenishes the soil with essential elements and add humus to the soil. Most animal manure is feces. Common forms of animal manure include farmyard manure (FYM) or farm slurry (liquid manure). FYM also contains plant material (often straw), which has been used as bedding for animals and has absorbed the feces and urine. Agricultural manure in liquid form, known asslurry, is produced by more intensive livestock rearing systems where concrete or slats are used, instead of straw bedding. Manure from different animals has different qualities and requires different application rates when used as fertilizer. For example horses, cattle, pigs, sheep, chickens, turkeys, rabbits, humans (sewage), and guano from seabirds and bats all have different properties. For instance, sheep manure is high in nitrogen and potash, while pig manure is relatively low in both. Horses mainly eat grass and a few weeds so horse manure can contain grass and weed seeds, as horses do not digest seeds the way that cattle do. Chicken litter, coming from a bird, is very concentrated in nitrogen and phosphate and is prized for both properties.

Animal manures may be adulterated or contaminated with other animal products, such as wool (shoddy and other hair), feathers, blood, and bone. Livestock feed can be mixed with the manure due to spillage. For example, chickens are often fed meat and bone meal, an animal product, which can end up becoming mixed with chicken litter.

In agriculture, poultry litter or broiler litter is a mixture of poultry excreta, spilled feed, feathers, and material used as bedding in poultry operations. This term is also used to refer to unused bedding materials. Poultry litter is used in confinement buildings used for raising broilers, turkeys and other birds. Common bedding materials include wood shavings, sawdust, peanut hulls, shredded sugar cane, straw, and other dry, absorbent, low-cost organic materials. Sand is also occasionally used as bedding. The bedding materials help absorb moisture, limiting the production of ammonia and harmful pathogens. The materials used for bedding can also have a significant impact on carcass quality and bird performance.

The effect of earthworm (*Eudrilus eugeniae*) on the bioremediation of used engine oil contaminated soil, amended with poultry manure, was investigated. Investigation into the effect of initial concentration of used engine oil, in soil, for earthworm inoculated samples showed that the biodegradation rate of used engine oil contaminant increased with increasing initial concentration of used engine oil. For initial used engine oil concentration of 5, 10, 15 and 20 g/kg soil, the drop in total petroleum hydrocarbon was found to be -16.91, 20.82, 34.68 and 36.28% respectively after 42 days of treatment. Schaefer (2001) investigated use of earthworms did not result in catalysis of the bioremediation process as the extent of biodegradation for the sample without earthworm was found to be higher than those of all concentrations of earthworm considered (5 – 20 worms/Kg soil). However, the rate of bioremediation was found to increase with increase in earthworm concentration (as earthworm concentration was increased from 5 to 20 worms per kg of used engine oil contaminated soil). Similarly when the rate of bioremediation in the without-earthworm but manually tilled and the without-earthworm and untilled samples where compared with samples inoculated with earthworm for bioremediation, both samples without worms recorded higher bioremediation rates.

Cow dung, also known as cow pats, cow pies or cow manure, is the waste product of bovine animal species. These species include domestic cattle (cows), buffalo, yak, and water buffalo. Cow dung is the undigested residue of plant matter which has passed through the animal's gut. The resultant faecal matter is rich in minerals. Color ranges from greenish to blackish, often darkening soon after exposure to air. The degradation of both crude and refined oils seems to involve a consortium of microorganisms, including both eukaryotic and prokaryotic forms. The most common genera known to be responsible for oil degradation comprise mainly *Nocardia, Pseudomonas, Acinetobactor, Flavobacterium, Micrococcus, Acinetobactor, Corynebacterium, Achromobacter, Rhodococcus, Alecaligenes, Mucor, Fusarium, Bacillus, Aspergillus, mycobacterium, Penicillium, Rhodotoruk, Candida and Sporobolomyces*. (Atlas 1981; Bossert and Bartha 1984; Atlas and Bartha 1992; Sarkhoh et al 1990) . Organic matter also improves may of the water holding capacity, aeration, pH, and ion exchange capacity (Brady and Weil 1998). These properties influence the indigenous microbial population may enhance their ability to degrade hydrocarbons and other C-based contaminants (Wellman et al. 2001). The incorporation of organic amendments affects soil enzyme activities because the added material may contain endo. or exocellular enzymes and may also stimulate soil microbial activities

(Goyal et al 1993). Okolo et al. (2005) uses poultry manure for the degradation of crude oil in contaminated soil. The soil amendment interacted in affecting crude oil degradation. This was optimal with a combination of poultry manure (2.0% w/w) + glucose (2.0% w/w) which yielded a crude oil degradation of 7.42±1.02% after sixteen weeks of incubation.

# CHAPTER 3

# METHODOLOGY

## 3.1 Sample Collection and Preparation

3.1.1 *For original lubricant oil contaminated soil*

The soil sample was collected near automobile shops where soil is contaminated with lubricant oil or oil filling station. Organic manure used for soil amendments for this study will be collected from site where cow dung / manure is dumped.

3.1.2 *Microcosm study*

The soil was collected from the site where no history of lubricant oil spillage. The soil was dried, meshed and sieve it from 2 mm size sieve. Autoclave the soil 2-3 times for complete sterilization and killing of microbes present in it.

The soil was amended with manure in different ratios like 5, 10, 25, 50 and 75%. The 5% amend contain 285 g soil and 15 g manure, 10% amend contain 270 g soil and 30 g manure, 25% amend contain 225 g soil and 75 g manure, 50% amend contain 150 g soil and 150 g manure, 75% amend contain 75 g soil and 225 g manure for making of one 300 g of sample.

The experimental samples will be set up in pots and monitored for a period up to 30 days in triplicate. The control sample will be composed of soil only and soil containing known amount of automobile oil. The 60% moisture was maintained in the pots by sprinkling water occasionally.


**Fig. 3.1 Experimental set up of microcosm study**

### 3.2 Estimation of Total Petroleum Hyrdocarbon by Chloroform

Automobile oil is extracted by using chloroform from the soil samples and quantitatively determined the absorbance at 520nm by using UV-VS spectrophotometer .

In this soil samples was air dried to constant weights and 5g were placed into small containers and 10ml chloroform was added. Residual oil was extracted by gently shaking the flasks for 2 min. Each extract was filtered with whattsmann filter paper in glass container, close immediately and analyzed for lubricant oil content. A standard curve of absorbance (520 nm) against varying concentration of lubricant in chloroform was drawn after taking readings from a spectrophotometer. The lubricant oil concentrations were calculated from the standard curve.

### 3.3 Study Microbial Monitoring

The study was conducted at room temperature and monitoring was performed on days 0, 7, 14, 21 and 28 days. To check the number of cells and degradation. Ten g of soil sample mixture was removed from the container and 10 ml of sterile saline and centrifuge the sample. The mixture was shaken on a vortex mixture for 5 min and then let particulates settle down. Aliquot 0.1 ml of sample and spread on nutrient agar plate.

#### 3.3.1 *Hetrotrophic Bacterial Count*

The total aerobic heterotrophic (TAH) bacterial population was estimated using the plate count method with nutrient agar as growth medium. The serial dilution prepared $10^{-1}$ to $10^{-6}$ dilution and $10^{-1}$ to $10^{-6}$ was surface spread on nutrient agar plates. Plates were incubated at $35^0$c temperature for 48 hrs after which the number of colonies formed was used to estimate the TAH bacterial population.

#### 3.3.2 *Isolation of fungi*

Serial dilution of manure sample were utilize for fungus

Dilutions$10^{-1}$ which was prepared for bacteria was utilized for fungus. Sample was spread on potato destrose agar and plates were incubated at $28^0$c for 48 to 96 hrs and observed the growth of fungus .

**3.4 pH**

Ten gram of soil and manure sample were dissolved in 100 ml of distilled water and flasks were shaken in incubator shaker (New Brunswick Scientific) for 2 hrs at 130 rpm. After 2 hrs sample kept at stationary condition until the sediments settled down. Check the pH by pH meter.

**3.5 Moisture:**

Soil and manure mixture ware placed in preweight petriplates. Sample were dried at 105$^{\circ}$C till constant weight were obtained. Percentage moisture of the mixtures were as by the equtaion.

$$Moisture\ \% = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100$$

**3.6 Chemical Analysis of Mixture of Soil and Manure:**

In soil chemical analysis, different chemical tests are there to check the soil chemical properties which are required like pH, conductivity, organic matter in soil, total nitrogen. The methodology of soil chemical analysis are given below:

3.6.1 *Conductivity*

1. Weigh 10 gm of sample and add to it 100 ml of distilled water.
2. Keep the suspension with continuous stirring for 15 min and leave it for over night.
3. Connect the instrument and calibrate the cell constant of the cell with 0.01 (745.6 mg KCl/L distilled water) and (0.1 M KCl) to instrument. Put the temperature knob also in the KCl solution.
4. After calibration dip the conductivity electrode into the suspension and record the result.

Dissolved minerals, gases and organic may produce aesthetically displeasing colours, taste and odorous to water. Conductivity measurements are the basic to evaluate the performance of the process of desalination, demineralization, distillation and many process plants (Rajor 2008)

3.6.2 *Organic matter*

1. Take 2 gm of soil (passed through a 0.2 mm sieve) in a conical flask and add 10 ml of 1N $K_2Cr_2O_7$ solution.
2. Add 20 ml of concentrated $H_2SO_4$, swirling the flask during addition, cool the contents of the flask for 30 minutes to complete the reaction at room temperature.
3. Add approximately 2 gm of sodium fluoride or 10 ml of orthophosphoric acid, 100 ml of distilled water and shake vigorously to mix.
4. Add 10 drops of the indicator diphenyl amine and titrate it with N/2 ferrous amm. sulfate till the colour changes from vilot to bright green.

3.6.3 *Total nitrogen*

Acid Digestion of Sample

1. Take 10 gm of soil rap into filter paper and put into a 500 ml of kjeldhal flask. Soak the sample with little water. Add 30 ml of conc. Sulphuric acid and shake by swirling for about 15 minutes.
2. Add 10 g of Hibbardi's mixture ( $k_2SO_4$, $CuSO_4$), 1 gm salicylic acid and 5 gm of sodium thiosulfate. Heat slowly till there is no frothing.
3. Raise the heat and continue digestion until the contents of the flask are grey or greenish yellow in color. Cool and add about 100 ml of water. Swirl well and transfer the contents to a 250 ml volumetric flask and make the volume upto the mark. Filter the contents of this flask for carrying out distillation.

Distillation

Take exactly 20 ml of 0.1 N $H_2SO_4$ into a 50 ml conical flask, add two drops of methyl red indicator and place under the delivery tube of the condenser in the distillation apparatus. Pipette 10 ml of the filtrate in the distillation flask add 10 ml of NaOH solution in this flask through a funnel connected through a tube to the distillating tube and distill the filtrate. Collect about 30 ml of distillate and when the distillation is over (tested by litmus), remove the receiver (conical flask) and then put off the burner. Titrate excess of acid in the receiver against 0.1 N NaOH until the colour changes from pink to yellow. Note the volume of 0.1 N NaOH used (Rajor 2008).

# Chapter 4

## RESULTS AND DISCUSSION

This chapter includes the results of experiments which included in remediation. The experimental study reports showed effects of manure rich in nutrients on the microbial utilization of petroleum hydrocarbons on lubricant oil polluted soil. The experimental samples mix with organic manure in different proportions were analyzed weekly for various parameters such as pH, moisture content, loss in crude oil and total heterophilic count. Culture dependent and culture independent approaches such as measuring soil respiration and enzyme activities, microbial counts, and molecular technologies may provide information about viable microorganisms presented in such environments (Van Hamme et al. 2003).

### 4.1 pH

The pH of lubricant oil contaminated soils with organic manure is given in figure . pH refers to the hydrogen ion concentration in soil which plays a vital role in decomposition process. At the start of the experiment, the pH of the all amendments including control was neutral in range. The pH was increased with increase of amends up to 10% till the end of the experiment where as at higher concentration of amends the pH was reduced from 8.33 to 8.23, 8.63 to 8.05 at 25% and 50% amends (table 1) respectively. The increase in the first three amends (5%,10% and 25%) could be from high metabolic activities possibly resulting in the production of intermediate metabolites in the organic manure systems (AMEH et al. 2012). The decreases observed in subsequent amends in all the treatment were attributed to the degradation of the organic manure and the hydrocarbons which may have resulted in the release of acidic intermediate and final products that probably lowered pH of the mixture. Generally pH in between 6.7-9.6 is more suitable for bacterial growth (Vincet et al. 2011).

The variation in pH is very less it may be due to the manure which was having its own alkaline pH.

**Table 4.1 variation in pH with different mixture.**

| Manure in soil (%) | No. of days | | | |
|---|---|---|---|---|
| | 1 | 14 | 21 | 28 |
| 0% | 8.43 | 8.49 | 8.45 | 8.77 |
| 5% | 8.34 | 8.39 | 8.5 | 8.69 |
| 10% | 8.25 | 8.29 | 8.43 | 8.5 |
| 25% | 8.33 | 8.30 | 8.23 | 8.61 |
| 50% | 8.63 | 8.37 | 8.05 | 9.17 |
| 75% | 9.18 | 8.86 | 9.2 | 9.58 |

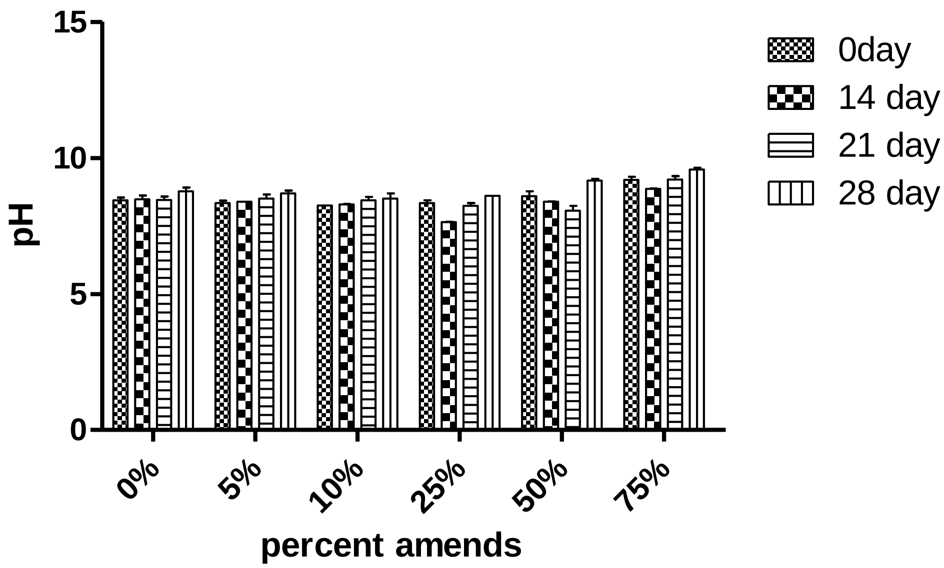

**Fig4.1 pH profile with different manure + soil mixture**

## 4.2 Moisture content

The moisture content in lubricant oil contaminated soil with organic manure amends is given in table 4.2. Generally, optimum microbial activity is achieved by the maximum water content that does not restrict oxygen diffusion(water logging condition inhibit the growth). The present study showed an increase in the moisture content with increase of amends and with the time. Water is crucial to transport of many microbial cells and therefore substrate colonization occurs. The optimum soil moisture in land forming of petroleum wastes was found between 30 - 90% .The soil amended with manure found maximum moisture 67.5% in 25% mixture where as the minimum 20.1% moisture in 10% amend. The moisture is a important factor for the growth of the bacteria as the nutrients also in the liquid form easily available to them. Microbes population in moisture containing soil and manure was found good in vibiality from rather then drysoil and manure.

**Table4.2 The moisture content in different mixture of manure and soil**

| Manure in soil (%) | Moisture (%) |
|---|---|
| 0% | 26.1 |
| 5% | 36.6 |
| 10% | 20.1 |
| 25% | 30.5 |
| 50% | 38.4 |
| 75% | 45.21 |

### 4.3 Loss of lubricant oil from contaminated soil with organic manure

A variety of technologies are available in the system to treat any type of contaminants with hazardous materials, this includes vapour extraction, stabilization, solidification, solvent extraction and thermal destruction etc. these techniquies are costly and also not given complete destruction of contaminents. On the other hand, biological treatment bioremediation appears better particularly to petroleum products which we have chosen. This technology is environmentally sound and can result in the complete destruction. This process does not require excavation of soil from ground.

The loss of lubricant oil in lubricant oil contaminated soils with organic manure amends is given below. The potential of the treatment option was shown by the percentage reduction of oil in the soil samples. The highest percentage oil reduction of 93.93% was observed in the sample supplemented with 50% manure. This was followed by the treatment with 25% (89.18%) and 10% (80.64%) .The samples without manure control and 5% organic manure amends have oil reduction 19.25% and 78.125% respectively. The loss of lubricant oil is increased with the increased organic manure amends and is significant ($r = 0.95$). This result shows that the nutrient supplementation enhances biodegradation rate which is in agreement with the works of Abu Go and Ogiji. It also showed that increasing % of manure in mixture of lubricant oil not only getting more of the nutrients but also has the more microbial consortia which degrade more oil. Desodun and Bagwu (2007) used the cow dung and poultry manure and pig manure for biodegradation of waste lubricating petroleum oil and observed that poultry manure induced the highest reduction oil concentration. Pala et al. (2006) observed that as bioremediation was done successfully still present which required some technical challenges.

The bioremediation by micro organism is the most promising technologies which is ecofriendly, cost effective and having potential ability to remediate on environment without potential ability to remediate on environment without causing much damage.

Abioye et al. (2012) also studied biodegradation of used motor oil in soil using organic waste amendments. He used brewery spent grain (BSG), banana skin (BS) and spend mushroom compost (SMC) for 84 days. The 92% degradation was observed with BSG. The nitrogen and phosphorous is essential for hydrocarbon degradation as microorganisms they required for their growth as well as for degradation.

The addition of organic and inorganic nitrogen rich nutrients (biostimulation) is an effective approach to promote the degradation application . Ijah and Antai 2003 reported partial degradation of hydrocarbons in the period of 12 months when 30 and 40 % crude oil was used with soil. Dadrasnia and Agamuthv (2013) also studied the effect of soil amendments on diesel fuel degradation and used three organic waste namely tea leaf , soy cake and potato skin for three months and observed 75, 81, 88% respectively in tea leaf , potatoleaves and soy cake.

93% degradation was observed when soil was supplemented with 75% manure and minimum with 10%. These results indicates that the effectiveness of microorganisms in degrading these oil and toxic chemicals depends on their natural, selected and tolerance to the percentage of toxic chemicals and oil present during the remediation process. Due to presence of microbes this technology become attractive which results in partial or complete biotransformation of many organic contaminants. This technology is believed to be cost effective, ecofriendly to the atmosphere (Agamuthu 2010). The diversity which is present in manure give wide range of their capabilities to synthesize or degrade organic compounds (Ramakrishnan et al. 2011).

The combined action of biostimulation through manure addition and bioaugmentation provided a good result for removal of total lubricant petroleum hydrocarbon from diesel contaminated soil (Taccari et al. 2012). Facundo et al. (2001) used bacterial consortium to degrade diesel oil in soil as well as in liquid and fond better results in liquid. The oil biodegradation in contaminated soil is dependent upon some factors such as creation of optimal environmental conditions, types of hydrocarbons in the contaminated matrix and its availability of oil, etc.

**Table 4.3 Degradation of TPH with time**

| Manure in soil (%) | No. of days | | | |
|---|---|---|---|---|
| | 1 | 14 | 21 | 28 |
| 0 | 0.026 | 0.021(19%) | 0.021(19%) | 0.02(23%) |
| 5 | 0.032 | 0.019(40%) | 0.018(43%) | 0.018(43%) |
| 10 | 0.031 | 0.018(43%) | 0.018(43%) | 0.017(43%) |
| 25 | 0.037 | 0.02(45%) | 0.02(45%) | 0.015(59%) |
| 50 | 0.033 | 0.015(54%) | 0.01(69%) | 0.002(69%) |
| 75 | 0.02 | 0.012(40%) | 0.007(65%) | 0.005(75%) |

***value in brackets denotes the percent reduction in TPHcontent.**

Table 4.3 showed that addition of 75% of manure the decrease in PAH'S was observed maximum in 28 days and minimum is on 14$^{th}$day after addition of manure. Adesodun and Mbagwc (2008) also observed biodegradation of lubricant by using poultry manure pig waste and cowdung and got good results of degradation in the same pattern PM>CD. Agamuth et al. (2013) also used organic waste to bioremediate hydrocarbon contaminated soil. they used sewage sludge and cowdung for 96 day and found 82% and 96% respectively degradation. The reason might be due to difference in the N and P content present in the organic wastes used.

**Standard curve**

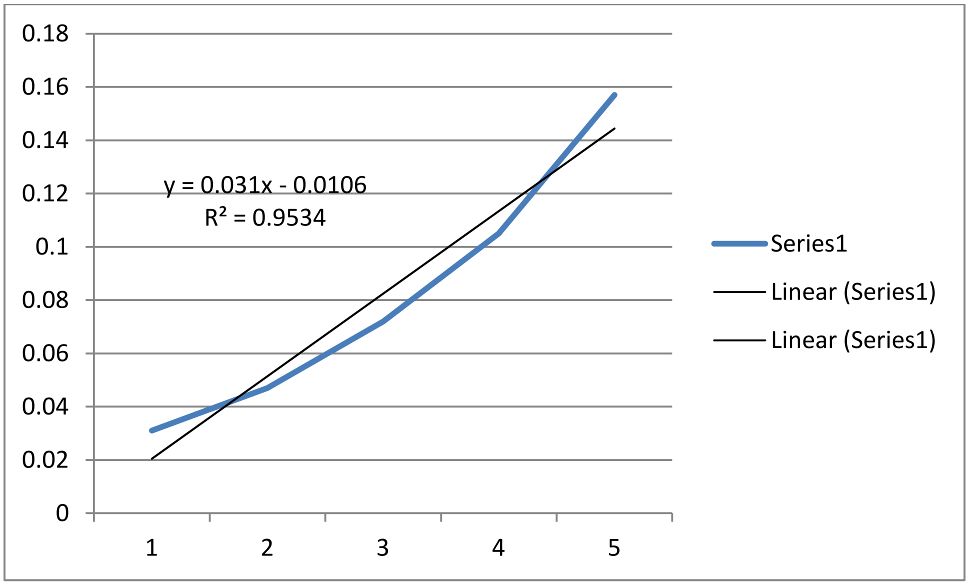


**Fig 4.3 Standard curve of oil with chloroform**

## 4.4 Total Heterotrophic Count (THC)

The liqiuid basal medium was used for the isolation of microbes which was composed of  as in table 4.

**Table 4.4 Different chemical composition for liquid basal medium**

| **Chemical** | **Quantity (g/L)** |
|---|---|
| Sucrose | 10 |
| $K_2HPO_4$ | 2.5 |
| $KH_2PO_4$ | 2.5 |
| $(NH_4)_2HPO_4$ | 1 |
| $MgSO_4.7H$ | 2 |
| $FeSO_4.7H_2O$ | 0.01 |
| $MnSO_4.4H_2O$ | 0.007 |

Lubricant oil was obtained from tractor workshop and was mixed 5g/kg (w/w) in soil uniformly mixed and allowed to be absorbed for 30-40 minutes. The different ratio of manure was mixed in soil.

The total count in control manure was found $5\times10^7$cfu/ml. which is quite higher when we added manure in different ratios in soil (contaminated) the total count was observed cfu/ml is given in fig 4.5. These results indicates that number of microbes keep on increasing after addition of manure.

**4.5 Microbial monitory**

The study was conducted at room temp. and monitoring was performed on days 0(or 1),14, 21, and 28. To monitor cell numbers and biodegradation 1 gm of soil + manure mixture was removed from the different containers after mix them thoroughly and then suspended in 10 ml of sterile saline. The mixture was vigorously shaken on a vortex mixture for 5 min. the sample was allowed to settle down for 5 min. 0.1 ml of supernatant sample were spread on agar plate for CFU count.

Gghazali et al 2004 used microbial consortium (bacillus and pseudomonas spp.) and found that both bacteria are good enough to degrade the oil content.

The total heterotrophic count (THC) in lubricant oil contaminated soil with organic manure amends is given in fig. the THC values are $10^9$, $231\times10^9$, $145\times10^9$ ,$228\times10^9$ cfu/g for lubricant contaminated soil amends of 5%, 10%, 25%,50% and 75% respectively for day 1 and after 14 days it was $261\times10^9$,$496\times10^9$, $520\times10^9$, $575\times10^9$, $1266\times10^9$ cfu/g respectively. These results showed that the soil 75% nutrient supplement gave the highest heterophilic soil 75% nutrient supplement gave the highest heterophilic bacterial count. The total heterophilic count is an indication of increase in microbial population which in turn increases ability to degrade lubricant oil in the presence of nutrient supplementation.

Namazi et al 2008 mixed culture (pseudomonas sp. Arthrobacter and myco bacterium sp.wereused for biodegradation of engine oil. With the mixed culture it observed that 47% of aromatic compounds was removed in 60 days of incubation. Ibekwe et al 2006 also observed organic nutrient on microbial utilization of hydrocarbon on crude oil contaminated soil. he checked both heterotrophic count as well as hydrocarbon utilizing bacterial count (HUB). The results of the study showed that addition of organic nutrients will enhance microbial utilization of hydrocarbons.

Ameh et al 2012used earth worm on bioremediation of used engine oil contaminated soil. the rate of bioremediation was found to increase with increase in earth worm concentration. (5 to 20 worms/kg of used engine oil.

The Total Heterotrophic Count (THC) in lubricant oil contaminated soils with organic manure amends is given in figure . The result showed an increase in the population size of microbes in the soils amended with organic manure compared to the one without the manure (control). This is due to the fact that organic manure on its own contains a diversity of organisms in addition to being a nutrient. THC values are $101\times10^9$, $231\times10^9$, $145\times10^9$ , $228\times10^9$, $459\times10^9$ cfu/g for lubricant oil contaminated soil amends of 5%, 10%, 25% and 50% respectively for day 1 and for day 14 THC values are $183\times10^9$, $293\times10^9$, $276\times10^9$ , $333\times10^9$, $1090\times10^9$ cfu/g .for day 21 THC values are $261\times10^9$, $496\times10^9$, $520\times10^9$ ,$575\times10^9$,$1266\times10^9$ cfu/g This shows that the soil with 75% nutrient supplement gave the highest heterophilic bacterial count. The total heterophilic count is an indication of increase in microbial population which in turn increases ability to degrade lubricant oil in the presence of nutrient supplementation.

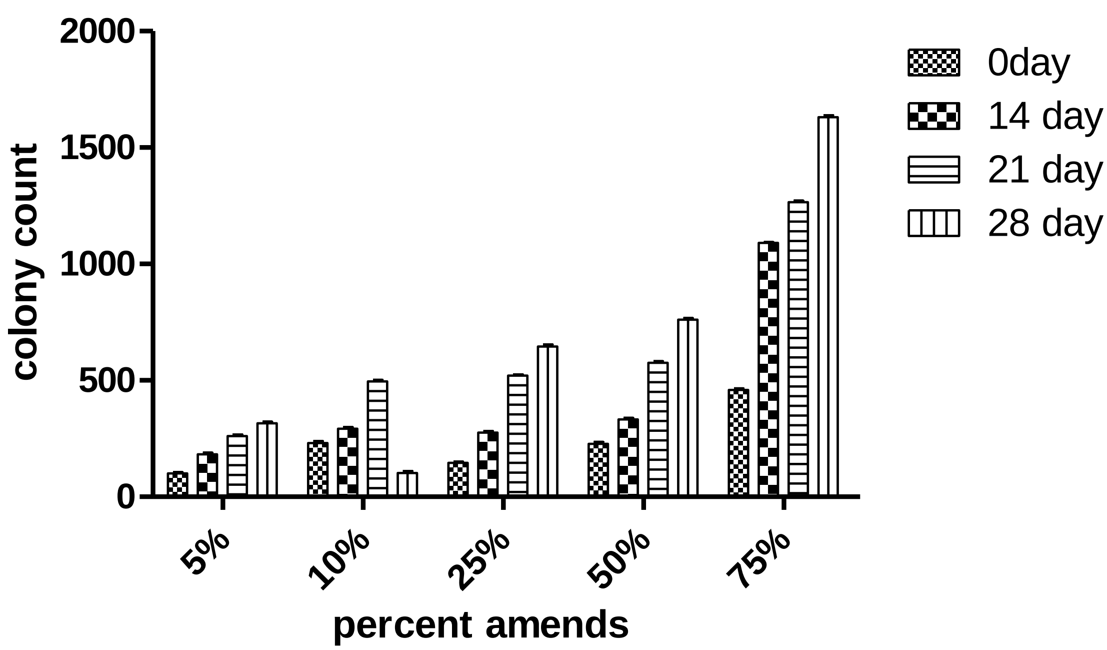


**Fig.4.5 Statically microbial growth with different time period.**

## 4.6 Contaminated soil sample

4.6.1 *pH*

The pH of the original sample contaminated with oil is 7.5 and when it was mix it with manure 50% the pH at zero day is 8.4 . after one month period the pH have slightly increases pH 8.6. whereas in control where no manure was added it was same as initially present.these results indigate that the pH does not play any major role in the degradation of lubricant oil or increase the microbial population but efficiency of bacteria increases at neutral pH .

4.6.2 *Moisture content*

Over all the month period the moisture content will be maintained to almost 50 to 70 percent so that for the growth of microorganism all the moisture condition are satisfied.

4.6.3 *Loss of lubricant oil from the lubricant oil contaminated soil with organic manure*

The loss of lubricant oil or degradation of oil should be check by chloroform method discussed earlier. The first day reading in spectroscope of the raw sample is 1.178 and with 50% amend the reading is 0.420. After one month of time period the reading of the raw sample still almost same and the 50% amend reading varying from 0.420 to 0.03. So the percent reduction in the presence of lubricant oil after one month period of time is 92%.

4.6.4 *Total heterotrophic count (THC)*

The first day reading in the raw sample was $105\times10^9$ and with 50% amend was $230\times10^9$. After one month period of time the raw sample reading does not vary and sample with 50% manure vary from $230\times10^9$ to $800\times10^9$ (70%).

Vineetha et al 2011 also observed 81.69% remrdial efficiency on the sixth week by using manure. Vineetha and Shibu 2012 observed 74% efficiency by using vegetable waste and sow dust in the ratio of 0.6:1 and 0.5:1 at neutral pH and 50% moisture.

4.6.5 *Chemical analysis of soil + manure*

The pH varies from 8.4 to 8.6 and the conductivity is 0.7 $mhos/cm^{-1}$.

# CHAPTER 5

## Conclusion

The study has shown that there is much potential in using organic manure as a method for treating with contaminated soil. Organic manure supplementation indeed enhanced the rate of biodegradation .The maximum removal of oil from amends was 75% after four weeks period at 75% amendments .The results show that nutrient supplementation enhanced the biodegradation of lubricant oil. However further studies are required to increase the efficiency of removal with organic manure at lower concentrations of amendments feasibility.

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