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Bioremediation of Crude Oil Contaminated Soils using Cow Dung as Bio-Enhancement Agent: A Review

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Abstract
Cow dung is an excreta obtained from bovine animals it is the most cheap and easily available bio resource available in our country cow dung is a dockyard of several microorganisms which are substantially worthwhile to the nature due to their capability to produce wide range of metabolites having instrumental importance Petroleum has developed to be the cornerstone of today's automobile society, now it has shown several threats to the present environment. By fault or accidentally several oil spills occur which actually can cause harm to the soil. Contamination of soil and water resources with petroleum oil and its products has become a serious problem due to carcinogenic and mutagenic compounds. Efforts are now focused on seeking potential remediation techniques for cleanup of petroleum hydrocarbons-contaminated soils in a cost effective and eco-friendly way. Various physical, chemical and biological remediation strategies have been used to restore contaminated soils.Cow dung is much rich in microflora *Acinetobacter, Bacillus, Pseudomonas, Serratia and Alcaligenes spp Stenalraphamomr and Pseudomonas species* were some of the common microbial isolates present in it. Cow dung also acts as rejuvenating agent as it provides source of Na, K, Ca and also when incorporated with any other animal manure showed best results.

Keywords
Cow dung, microflora, Physico chemical Factors, Animal Wastes, Hydrocarbons

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1. INTRODUCTION

1.1 Crude oil Contamination to the soil

The major wetlands and river deltas usually comprise of the oil and petroleum release fields this crude petroleum possess a potential to distort the physical structure and may cause several damages to the ecosystem in run and other nature associated functions. The marsh soil which is near to the area of oil wells have hydrocarbon content more than the nearby soil area significantly higher than those in the adjacent control marsh. Soil water contents in oil-contaminated marshes are negatively correlated with soil temperature and are significantly lower than those in the control area, especially in fall. The rise in pH upto 8.0 is observed in the area where the pigments are released. It also lowers the amount of Phosphate content in the soil. The amount range of total organic carbon are significantly different among

sampling sites. Therefore, crude oil contamination could potentially alkalinize marsh soils, adversely affect soil fertility and physical properties, and cause deterioration of the marshes **(Ying *et al.,* 2013).**

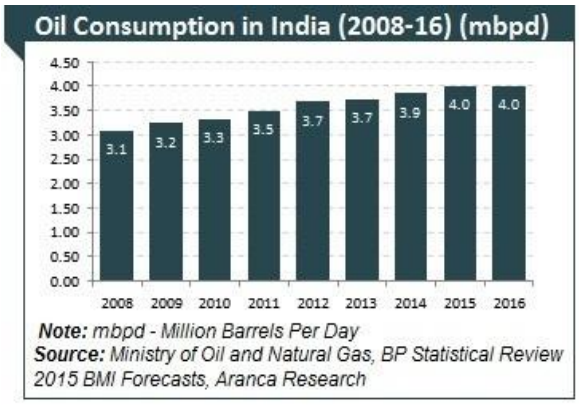
All activities surrounding the oil sector such as exploration, drilling, transportation, refining and consumption of oil and its associated products result in the spilling of oil and refine products into the environment. In 2013 alone, the NNPC reported a total of 2256 line breaks on NNPC pipelines resulting in a loss of 181.67 million tonnes (mt) of petroleum products worth about N21.48 billion, with 34 cases of fire incidents during the year under review **(NNPC 2013).**Developing an effective strategy for the removal of petrochemicals from contaminated environment is one of the major challenges facing developing countries including in her quest for economic development **(Chaineua *et al*.,2013).**

1.2 Bioremediation in removal of Crude oil contaminants

Bioremediation is the process of using an organism to neutralize or remove contamination from waste. No chemicals are used in the process of bioremediation as it is so important fact about it, although it may use an organism which can be threat under certain circumstances. A gross, but simple explanation of bioremediation is the use of maggots like organisms in wound care control. Wounds that have contamination can have maggots introduced to them. The maggots then eat the contamination, allowing the wound to heal correctly. That is a form of medical bioremediation but there are many other types that are used to control different waste contamination **(Ofoegbu *et al.,* 2015).**

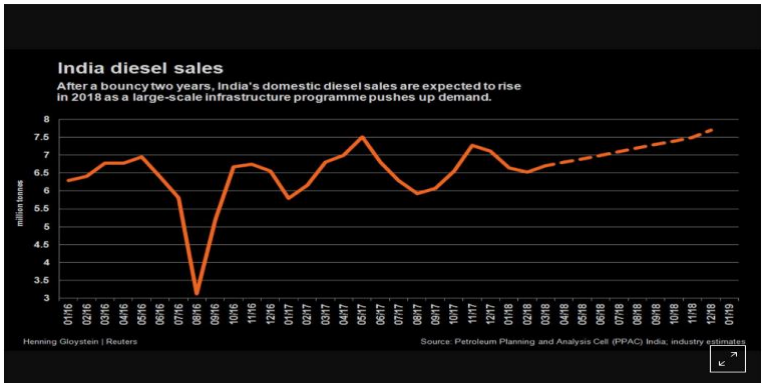
Bioremediation can also be used to deal with the environmental pollution that is a serious threat, the oil spills are causing soil contamination and a great impact on flora and fauna **(Jain *et al.,* 2011).**

As per below data, India Consumes around 4 Million barrels per day of oil. This is general oil consumption which includes all the petroleum products like Petrol, Diesel, Kerosene etc.



Oil Consumption between the year of 2008 to 2016 as confirmed by the Natural of Oil and Natural Gas

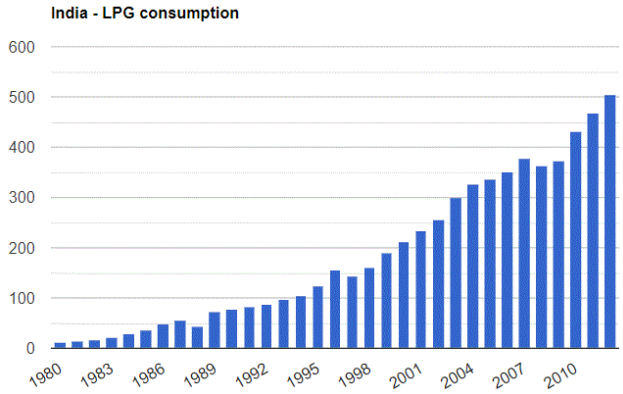
Going by the year on year trend in the graph, we can expect the consumption for the year 2017 not more than 4.1 mbpd . 40% of fuel consumption of India is Diesel and average consumption of Petrol is about 12%. But it had the highest average growth rate among Oils at 15.56% during March 2016(3 months average). The share of consumption of Petrol will not be more than 14 % now. So, 14% of 4.1 mbpd is 0.574 mbpd which would be 574000 Barrels Per Day which is 91258707.29 liters per day.



Source: A data from Indian Diesel sales -2018

Data mentioned by **Petroleum Analysis and Planning cell** estimated that total Diesel Consumption has been very high in the past few years which has been a threat to the soil for their contamination.

So, to Summarize approximately petrol sold in India is, 91258707.29 Liters per day 3802446.13 Liters per hour 63374.10 Liters per minute 1056 Liters per Second Besides, petroleum gases are major source of fuel for domestic cooking. The **U.S. Energy Information Administration provides data of LPG consumption for India from 1980 to 2012**. The average value for India during that period was 185.46 thousand barrels per day with a minimum of 12.19 thousand barrels per day in 1980 and a maximum of 506 thousand barrels per day in 2012.



Source: Indian LPG consumption from the year 1980-2010

This concludes the overgrowing demand for petroleum hydrocarbon as the major energy source of domestic cooking and lighting as well as motor fuel has evidently led to disturbing cases of crude oil spillages. The presence of heavy metals in some environments has, therefore, been leading to petroleum prospecting and mining as well as oil spills. These metals, however, can disrupt various cellular processes and their effects are often concentration dependent and also differ according to their individual toxicity. There are a very few reports of isolation of petroleum hydrocarbon degrading bacteria. Some scientists conducted experimentation and examined the effectiveness of *Pseudomonas* spp. Culture **(Pradeep *et al*., 2011**).

2.1 Cow dung as major Bio-enhancement agent

Microflora of Cow dung in Bioremediation of crude oil Contaminated Soil

Pseudomonas species were noticed to degrade high amount of aliphatic compounds. Hydrocarbon degradation through the use of microbial seeders has been a colossal and ultimate natural approach to detoxify/cleanup of petroleum hydrocarbon pollutants from the affected soil. In some studies, microorganisms were isolated from animal waste were introduced into crude oil polluted soil. The activity of these microorganisms on crude oil was monitored. The result revealed that these bacterial cultures isolated from animal wastes were expressing abilities to utilize crude oil as their sole carbon source and can be very effective when applied as microbial seeders to be used as bioremediation approach on crude oil contaminated environment

There have been several studies which concluded the benefits of cow dung isolates, both bacterial and fungal, for depletion of total petroleum hydrocarbons to null in polluted mangrove environment. Cow dung also have diverse population of microorganisms such as *Acinetobacter, Bacillus, Pseudomonas, Serratia and Alcaligenes spp*. which makes them efficient for depletion of pollutants (**Orji *et al.*, 2012)**.

Applying the scientific findings, animal waste (Chicken droppings and Cow dung) are recommended for the elimination of crude oil pollutant as bioremediation approach on polluted soil and rejuvenation/maintenance of natural land value for farming purposes **(Yadav and Thakrey, 2013)**.

The metagenomic 16s rDNA library contained 68% clones representing unculturable bacteria that belonged to phyla ***Bacteroidetes, Firrnicutes and Vemrmicrobia.*** All clones within the attempt was made by (**Girija *et al.* 2012)** to find the diversity of microbes present in cow dung through a culture-independent, 16s rDNA sequencing approach.

The predominant phyla found in the study were *Bacteroidetes, Firmicutes and Proteobacteria*. Members of these phyla are found to have an

efficient degrading activity of complex organic matter like cellulose, lignin, chitin, xylan, etc. Therefore, results of the present investigation justify the use of cow dung in composting. This study also detected *Acinetobacter, Bacillus, Stenalraphamomr and Pseudomonas species.* All of which have already been reported as IAA and siderophore producers. Many Acinetobacter and Pseudomonas species have been reported to have nitrogen fixing and phosphate solubilizing activities, thereby imparting plant growth promoting activity of cow dung as observed by farmers.

Several genera of bacteria identified in the study (Bacillus & Pseudomonas) are known for antagonistic properties against bacteria and fungi. This results justifies the use of cow dung is being used as purifier in various religious practices and for disease suppression in organic *farmingphylum Preoteobacteria* were culturable. Many strict anaerobes requiring specialized conditions to grow *(Ciostridmnz, Bacreroides, Alisripes, Ruminococcus, Anaemvorax & Akkermansia)* were also detected. Cultuxe-based diversity analysis can detect only easily grown *E colt', Lactobaeilli, Streptococci and Bifidobecteria*, but may fail to detect fastidious and anaerobic bacteria. Therefore, the study revealed the superiority of culture-independent rmtagenomic approach as a powerful tool for elucidating the diversity of animal microbiomes **(Gupta *et al*., 2016)**. Sequencing of more clones may give a clear and complete idea of cow dung microbiota. Furthermore, detailed studies are needed to be done to elucidate microorganisms which show no similarity to any identified culturable non-culturable microbe.

The varied mean count of 1.54 × 104 cfu/g was recorded by cow dung sample for petroleum hydrocarbon utilization and 1.69 × 105 cfu/g for total for total heterotrophic fungi mean count. It was found that Bacillus had 100% occurrence and hydrocarbon clastic potential followed by *Psuedomonas* and *Mirococc*us with 85% occurrence. *Klebsiella* and *Seratia sp.* had the lowest percentage occurrence of 35% and 25% respectively **(Udgire *et al.,* 2015).** The use of animal waste (Chicken droppings and Cow dung) is an innovative approach in bioremediation of crude oil due to the natural abundance of nutrients and stable pH value for microbial proliferation to facilitate the biological remediation process.

Therefore, to maintaina fertile soil (farmland) polluted with crude oil it is recommended to be with proper supply of Nitrogen and Phosphorus necessary for plant growth, animal waste such as chicken droppings and cow dung are benificial to serve as nutrients and enhance the proliferation of organisms that are capable of degrading and utilizing crude oil as their main hydrocarbon source **(Panda *et al.,* 2013).**

2. Cow Dung-Enriched source of nutrients.

2.1 Physico-Chemical factors affecting the swift bioremediation by microflora of Cow dung

A research was conducted a research work which revealed that animal wastes could be used in bioremediation of crude oil pollute soil for enhanced remediation of polluted agricultural soil. They isolated microorganisms from animal waste and introduced into oil polluted soil. Their effects were carefully monitored. The result of the physico-chemical parameter of polluted soil monitored after two weeks showed increase in the soil pH from (5.67-5.99) and decrease in available phosphorus from (29.99-3.00) and total nitrogen from (0.27-0.06), exchangeable Ca from (2.82-2.46), Mg (1.20-0.98), Na (0.05-0.04) and K (0.08-0.06) in the crude oil polluted soil when compared to values obtained from unpolluted soil which served as control. The clay (15.02) and silt (19.75) particles of the polluted soil were higher than those of the unpolluted soil clay and silt. The physicochemical inspection of polluted soil amended with animal wastes from start day (Day 1) to week 12 **(Inieke *et al*., 2018).**

The rise in number of colonies signified an increase in the electrical conductivity, total Nitrogen, Ca, Na and K when compared to the values obtained from untreated polluted soil. The experimental design for soil treated with crude oil. At the end of the incubation period, 85.78% and 56.81%, of crude oil was degraded in soil treated with animal waste (Chicken droppings and Cow dung) respectively.

Cleaning and remediation of hydrocarbons from an oil spill is difficult and the techniques for cleansing up an oil spill are majorly affected by a numerous factor such as the type of oil spilled, the temperature of the water body, and the types of shorelines and beaches involved. A number of techniques and technologies have been innovated for spreading of oil spills in marine shorelines and freshwater environments. Many mechanical and chemical can be applied to remediation of the spills (**Zhu *et al.,* 2001**)

Cow dung amended soil was found to have improved soil physiochemical characteristics that featured very quick adaptation by the microorganisms in the contaminated soil **(Agamuthu, 2013).**

2.2 Hydrocarbon Reduction in terms of exhaustion by micro flora

The enhanced oil degradation observed with cow dung and chicken droppings indicated that the soil

amendments induced enhanced crude oil degradation. Maximum oil reduction of 320.74ppm, reductive to the unamended control was observed from treated sample with cow dung. As observed, the total mean heterotrophic count of bacteria presents in polluted soil treated with wastes ranged from 1.56-3.31 × 108 cfu/g. The total mean count of hydrocarbon degrading bacteria ranged from 1.14-1.66 × 104 cfu/g. The total heterotrophic count of unpolluted soil sample was 4.8 × 108 cfu/g and the values from heterotrophic count from soil sample were comparatively higher than that of hydrocarbon degraders 0.6 × 104 cfu/g **(Inieke *et al.*, 2018).**

Cow dung slurry mixed in the ratio of 1:10 or 1:25 is able to degrade the rural, urban and hospital wastes, including oil spillage to five basic elements **(Gurpreet and Jagdev 2011).**

2.3 Amount and Duration of Cow dung required

A research work conducted by **(Osazee *et al.,* 2015)** explained that bioremediation of crude oil with the help of microbes present in cow dung. They performed experiments on microbial culture on crude oil for 6 weeks on three samples of soils collected and experiments were performed which showed the degree of total fungal populations, hydrocarbon utilizing fungal populations, and the quantitative hydrocarbon losses were also determined at different weights of Cow dung. True oils were mixed with different soil samples collected from different locations and labelled. The contaminated and uncontaminated soils were allowed to stand under natural environment for two weeks before application of different levels of cow dung. Well in this period, the soil samples were provided with water at interval of two days.

A collection of 36 bowls with soil were mixed with crude oil and 12 bowls without crude oil contamination.

Two weeks past of contamination, cow dung was carefully weighed into the bowls containing the crude oil at various weights (control, 30g/kg, 60g/kg and 90g/kg of soils). The cow dung was crushed before use. Soil samples were replicated 3 times and arranged in completely randomised design. The whole samples were monitored. The mean total aerobic fungi present in the samples at the initiation of the experiment and subsequently at 1-week intervals for each of the treatment procedures were estimated by means of spread plate method with Potato Dextrose Agar (PDA) as medium.

The results demonstrated that cow dung at the different weights tested was effective in biostimulation of fungal species in crude oil contaminated soil resulting to related increase in microbial population. Hence, priority should be given to the utilization of optimum application levels as the results of this study indicated that biodegradation respond to differences in treatment procedures for soil quality similar to that which was used for this study.

2.4 Accompanying of Cow Dung with different Animal Manure

For better results researchers found a way in which they blended cow dung along with the poultry manure, rabbit waste, and Goat manure.

The effect of poultry droppings along with cow dung on bioremediation of crude oil-polluted soil has been experimented by numerous researchers **(Williams et al., 1999; Ugochukwu et al., 2016).** In a study, it was reported that poultry droppings can be used as a good remediation material in the reconstruction of a crude oil-polluted earth crust and it is also a potential source of nutrients for microbial activity and various microbes capable of utilizing hydrocarbons as source of carbon and energy **(Ugochukwu *et al.,* 2016)**.

Ecotoxicity assessment involving seeds of bean seed (Viciafaba) were used to evaluate the proportion of contaminant removal in the amended soil with poultry droppings and a germination index of 95% was observed in the 50 % amended option only. In a similar study, the remediation of soil contaminated by the petroleum compounds were found to be significantly ($P< 0.05$) enhanced when provided with poultry litter (pelleted or nonpelleted) in concentrations of 10% soil volume **(Williams *et al.,* 1999).**

According to (**Hamid *et al.* 2005)**, the introduction of chicken manure as a nitrogen source may be necessary to increase microorganism populations at a hydrocarbon contaminated site. An experiment of chicken-dropping for oil spill remediation was performed **(Ijah and Antai, 2003)** and the observations indicated that chicken droppings enhanced degradation of the crude oil present in the soil environment. The amendment increased the acidity (pH 5.7) of the crude oil-polluted soil to alkaline (pH 7.2) within 16 days. The study demonstrated that bacteria in chicken manure ware able to decompose 50 percent more crude oil than soil lacking the amedment **(Bello *et al.,* 2009).**

Yet still rabbit manure has not been implemented in bioremediation but research says Although there is not more information on the application of rabbit droppings in the bioremediation of hydrocarbon contaminated soils, rabbit manure is found to contain dexterous bacterial community capable of decomposing different types of xenobiotics such as polychlorinated biphenyls (PCBs), pharmaceutical

components and other hydrocarbon derivatives **(Tharakan *et al*., 2006; Zeng et al., 2015).**

Solomon *et al.* (2015) had earlier provided info on the positive effect of rabbit droppings in improved biodegradation of the heavy proportions of total petroleum hydrocarbons in a weathered crude oil contaminated soil environment.

Manure from goat-The effect of goat droppings in a bioremediation study involving sawdust, yam peel and a mixture of cow dung, goat dung and poultry dung (alone or in combinations) was statistically not significant **(p < 0.05),** implying that goat droppings had a similar effect as other bulking agents. The system proposed here takes advantage of the organic wastes bulking properties as well as the autochthonous microorganism and their metabolic activity to efficiently degrade petroleum hydrocarbons **(Samuel and Lukuman, 2013).**

CONCLUSION

The clearance of Lithosphere by petroleum is the problem of an hour. A better understanding and machining up of essential phenomena for biodegradation has high ecological significance that is dependent on the alternative source of allochthonous hydrocarbon degrading bacteria from farm waste such as cow dung. Several research work revealed that animal wastes could be used in bioremediation of crude oil pollute soil for enhanced remediation of polluted agricultural soil. Fence bacterial isolates obtained from animal waste (Cow dung) possess biodegradation potentials and expressed competence in utilizing crude oil as a carbon source. The bacterial isolates with crude oil degrading potentials obtained were *Psuedomonas sp.* (being the maximum), *Micrococcus sp.* (being at the next number) and *Bacillus sp*. (complete interaction with the atmosphere) *Klebsiella and Seratia sp.* Had the lowest percentage occurrence in the soil, although their pathogenecity could also become significant factor. The experimental analysis unleashed that these bacterial isolates derived from cow dung microflora expressed abilities to utilize crude oil as a sole carbon source and can be very exclusive when applied as microbial seeders to serve as bioremediation approach on crude oil contaminated environment. Applying this scientific research, animal waste –Cow dungs are recommended for the elimination of crude oil pollutant as bioremediation approach on polluted soil and rejuvenation/maintenance of natural land value for farming purposes.

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