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Cow Dung as a Bioremediation Agent to Petroleum Hydrocarbon Contaminated Agricultural Soils

T. M. Neethu[1*], P. K. Dubey[1], A. R. Kaswala[1] and K. G. Patel[1]

[1]Department of Soil Science and Agricultural Chemistry, Navsari Agricultural University, Navsari, Gujarat – 396450, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author TMN decided the topic, collected the reviews and wrote the first draft of the manuscript. Author PKD collected more reviews on the topic and changes on the manuscript had done according to his logic. Author ARK managed the literature searches and rearranged the contents. Author KGP collected relevant literatures on various topics. All authors read and approved the final manuscript.

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ABSTRACT

Petroleum derived products are very important as they are energy source and it is prone to accidental spill regularly during the exploration, production, refining, transport and storage. The characteristics of the hydrocarbon content of the petroleum mixture influence the degradability of individual hydrocarbon components; the simpler the hydrocarbon structure the easier its biodegradability and the complex the hydrocarbon structure the harder its biodegradability. Furthermore, the order of biodegradability of hydrocarbon is alkanes > alkenes > alkynes > aromatics. Bioremediation technologies are effective techniques to mitigate many organic and inorganic contaminants such as hydrocarbons, halogenated organic compounds, halogenated organic solvent, non-chlorinated pesticides and herbicides, nitrogen compounds, radionuclides, heavy metals. Bioremediation is having different strategies like an exploration of indigenous microbial populations, bio-stimulation, temperature, soil pH, bio-augmentation, phytoremediation, photo-degradation, phyto-volatilization and phyto-stabilization. Cow dung, excreta of bovine animal

**Corresponding author: E-mail: neethutm999@gmail.com, neethu999@gmail.com;*

is a cheap and easily available bio-resource on earth. Many traditional uses of cow dung are already known in India. Cow dung harbors a diverse group of microorganisms that may be beneficial to humans due to their ability to produce a range of metabolites. Nowadays, there is an increasing research interest in developing the applications of cow dung microorganisms as a bioremediation agent to hydrocarbon contaminated soils. Microorganisms capable of degrading hydrocarbon pollutants have been identified and isolated from cow dung. These organisms include; *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Proteus kleibsilla, Aspergillus* sp., *Rhizopus and Penicillium.* Therefore, cow dung is an effective, economical and eco-friendly bioremediation agent which can lead to the complete mineralization of hydrocarbon. The post remediation assessment of residual hydrocarbons in contaminated soil can be done with gas chromatographic fingerprinting technique and phytotoxicity bioassay.

Keywords: Bioremediation; bio-augmentation; cow dung; hydrocarbon.

1. INTRODUCTION

Petroleum derived products are the major source of energy for our vehicles, industry and daily life. Due to its importance as an energy source, it is prone to accidental spill regularly during the exploration, production, refining, transport and storage. The contamination of soil by crude oil and petroleum derived product has become a serious issue of global concern because of the potential consequences on the ecosystem and human health [1]. The characteristics of the hydrocarbon content of the petroleum mixture influence the degradability of individual hydrocarbon components; the simpler the hydrocarbon structure the easier its biodegradability and the complex the hydrocarbon structure the harder its biodegradability [2]. Furthermore, the order of biodegradability of hydrocarbon is alkanes > alkenes > alkynes > aromatics. They are highly persistent in the environment, toxic and results in significant health risks to human [3]. These contaminants mainly affect soil biological activity *i.e.*, soil microbial biomass and enzymatic activity. So, soil microbial activity is commonly used to assess the disturbed soil. Soil enzyme activity has been considered as a parameter which provides a biological assessment of soil function. The harmful effects of oil in different environments has led to the need to develop simple adoptable remediation techniques for petroleum products polluted sites using different simple and affordable methods, which may include physical, chemical and biological processes [4]. The physical methods of incineration or dig and dump in secure landfills [5,6], as well as the chemical method which involves the use of thermal and solvent treatment have been extensively reviewed [7,8].

Global production of crude oil is estimated at more than twelve million metric tons annually, it has been reported that 1.7-1.8 million metric tons [9] of petroleum contamination is treated via chemical and physical technologies which are expensive compared to the bioremediation. These methods are however expensive when contaminated areas are large [4] and it may pose possible collateral destruction of the site material or its indigenous flora and fauna [10,11]. Bioremediation processes that employ the use of microorganisms to degrade environmental contaminants [12,13,14], have also proved effective and could be used to accomplish both effective detoxification and volume reduction. Bioremediation is mainly based on the ability of microorganisms to destroy or degrade organic compounds or minimize the concentration of these materials by the detoxification or mineralization of the organic pollutants to carbon dioxide and water. Bioremediation technologies are an effective technique to mitigate many organic and inorganic contaminates [15] such as hydrocarbons, halogenated organic compounds, halogenated organic solvents, non-chlorinated pesticides and herbicides, nitrogen compounds, radionuclide, heavy metals. The advantage of this remediation process over physicochemical remediation method is that it is believed to be non-invasive and relatively cost-effective [16]. Bioremediation processes could be enhanced either by addition of commercial microbe cultures (bio-augmentation) [17,18,19] or by nutrient enrichment (bio-stimulation) of the natural microbial population [13,14]. Numerous laboratory studies on the use of fertilizer to enhance oil biodegradation by naturally occurring microbes have concluded that fertilizer use has the potential as a treatment technique for removing hydrocarbon in an impacted area [20]. However, several components of fertilizer are toxic to humans and other organisms even at certain concentration. Secondly, nutrient concentration can inhibit the bio-degradation activity. Several authors have specifically reported the negative effects of a high NPK level on the biodegradation of hydrocarbons [21,22]. According to Hoff [23], microbes preferred to utilize organic components of the fertilizer instead of the oil.

Under these circumstances, there is need of eco-friendly bioremediation techniques. Animal manure is nutritionally rich in energy, protein, mineral and vitamins [24], which can help in the improvement of soil properties, especially farmlands, without any potential health risk on living biota. Cow dung is a cheap and easily available rich source of microflora. According to Ayurveda, Gomeya/cow dung is not a waste product, but it is a purifier of all wastes in the nature. When spread over urban and rural waste in solution form (1:10–1:25 solution), it biodegrades the waste in time. It is a “gold mine” due its wide applications in the field of agriculture, energy resource, environmental protection, and therapeutic applications [25]. Many traditional uses of cow dung such as burning as fuel, mosquito repellent and as a cleansing agent are already known in India. Cow dung harbours a diverse group of microorganisms that may be beneficial to humans due to their ability to produce a range of metabolites. Nowadays, there is an increasing research interest in developing the applications of cow dung microorganisms for remediating the hydrocarbon contaminated soils. Therefore, in this chapter the bioremediation by using cattle manure, especially cow dung in an effective way is reviewed.

2. STRATEGIES OF BIOREMEDIATION

These strategies always have been used in both aquatic and terrestrial environments. This strategy has been promoted over other methods (physical/chemical) for clean up because of the reduced risk of environmental impact [26,27].

2.1 Exploitation of Indigenous Microbial Populations

Bioremediation strategies include the usage of indigenous microbial populations in the remediation of contaminated sites. This involves the utilization of already existing microorganisms in a given environment to achieve successful remediation of a contaminated site. It has been established that these indigenous microorganisms are ideal candidates for use in the bioremediation of hydrocarbon pollutants [28]. The presence of a large number of microorganisms is an advantage at the start of the process.

2.2 Bio-stimulation

It involves supplementing the contaminated soil to change the physical state of the contaminant, thereby converting it to a more bio-available form [13]. The microorganisms involved may require supplementing the bio-stimulation conditions. This enables them to metabolize the pollutant. The bio-stimulation conditions include oxygen level, temperature, pH, presence of water, soil moisture, number and type of organisms present and the presence of heavy metals and salts [29, 30]. Petroleum degradation by microorganisms can occur in an aerobic or anaerobic condition [31]. However, the rate of degradation is faster in aerobic than in anaerobic condition and so the supply of oxygen is needed to maintain aerobic condition.

2.3 Temperature

Temperature is another factor that plays important role in biodegradation of petroleum hydrocarbon, firstly, by its direct effect on the chemistry of the pollutant and secondly, by its effect on the physiology and diversity of microorganisms [4].

2.4 Soil pH

Soil pH will also affect both the growth and the solubility of compounds. A slight alkaline pH of seawater seems to be favorable for petroleum hydrocarbon degradation but liming of acidic soil from pH 7.8 to 8.0 had a definite stimulatory effect [4]. In certain cases, hydrocarbon contamination may be associated with high level of heavy metals, which may inhibit microbial growth depending on the concentration and type of metals.

2.5 Bio-augmentation

This involves the addition of some selected non-indigenous microbial population to the soil to speed up degradation [32,13]. Bacteria are not only the microorganism used as they can grow under low water condition as well and are present in the soil and water. It has been reported that this technique has the advantage of introducing naturally developed populations cultured outside the soil. This technique has been shown to enhance the degradation of pentachlorophenol, atrazine and chlorobenzene (Armstrong *et al.,* 1995). Fungi species that have been used to bio-augment soil include

Methylosinus trichosporium and *Cladophialophora* sp. [33]). However, some researchers argue that bio-augmentation can only be effective in the laboratory but not in the field. The addition of microbe did not increase biodegradation because foreign strains of bacteria failed to compete with the indigenous population. Some of the possible factors responsible for bio-augmentation failures include the fact that the concentration of contaminants may not be sufficient to support growth; environment may contain substances that inhibit growth, predation by protozoa and that the introduced microbe may not be able to penetrate the soil to reach the contaminant. More recently, bio-augmentation has had more success using activated soil rather than pure culture. The activated soils are those soils containing indigenous microbial populations recently exposed to the contaminants. This technique has the advantage of introducing naturally developed population, not cultured outside the soil.

2.6 Phytoremediation

This involves the use of plants to extract or detoxify pollutants through physical, chemical and biological processes [34,35,36]. The use of plants for bioremediation is a welcome phenomena and it has the advantage of providing aesthetically pleasing ecological options. It has also minimal disruption of the topsoil and it can offer the possibility of recovery of metals. Phyto-remediation is inexpensive and very effective with low levels of mixed contaminants. Some plants have been reported to be used in phyto-remediation. Such plants include Dictyledon (*Thlaspi caerulescons, Brassica junica*), Grasses (*Vetiveria zizaniodes*), Fern (*Pteris vittata*) and some aquatic plants (*Azolla pinnata*) [37,38] and *Elicine indica* [27].

2.7 Phyto-degradation

Phyto-degradation is another strategy that involves the uptake and degradation of organic compound.

2.8 Phyto-volatilization

It involves the volatilization of pollutant into the atmosphere. Example of such plants includes Indian mustard (*Brassica juncea*) [28].

2.9 Phyto-stabilization

It is the transformation of species of toxic molecule into less toxic species (Cr^{6+} or Cr^{3+}) and involves plants such as *Zolium perenne*.

3. THE USE OF ANIMAL MANURE IN REMEDIATION OF HYDROCARBON CONTAMINATED SOILS

The animal manure has been reported to help in enriching the soil contaminated with hydrocarbon pollutants. Okoh [4] reported that the organic manure binds rapidly to the soil particle, and this facilitates the movement of the pollutants through dirt, when natural events like rain occur. In recent studies, animal manure has been used to enhance biodegradation of contaminated soil. Microorganisms capable of degrading hydrocarbon pollutants have been identified and isolated from animal manure. These organisms include; *Micrococcus sp, Bacillus sp, Pseudomonas sp, Enterobacter sp, Proteus kleibsilla, Aspergillus sp, Rhizopus and Penicillium* [39].

3.1 Value of Animal Manure in Remediation of Oil Polluted Soil

Heavy metals such as mercury, lead, zinc, chromium, nickel, cadmium and arsenic, which are highly toxic to human and agricultural soils, were recorded at higher levels in the spent oil polluted soils indicating that they are released into the environment through inappropriate disposal of the spent motor engine oils. The microbial population of the polluted soils compared to the unpolluted soil was also reduced probably as a result of nutrient imbalance created by spent engine oil pollution. Thus, the result of uncontrolled and unregulated dumping of waste like spent engine oil is excessive pollution of the immediate environment. Cattle manure can enhance biodegradation of spent engine oil-polluted soils. The nutrient content of the soils, which were severed due to pollution, were restored. This is because the animal dung contains high nutrient composition and so they provided the polluted soil with nutrient element, needed by both the endogenous microbes and those supplied by the different animal dung for their bioremediation activities. This also helped different microbial species found in the soils to proliferate for ultimate utilization of the spent engine oil. Nutrient supplements in the form of animal dung mainly cow dung caused a reduction in metals such as Zn, Cd, Ni, Pb, Ar, Cr, and Hg, which were made abundant in the polluted soil. So, it is very evident that cattle dung can process mixed culture of petroleum degrading microbes and the addition of these increase both the population and diversity of both bacteria and fungi isolates in the polluted environment to enhance remediation [40,41].

3.2 Characteristics of Cow Dung

Cow dung is superior in terms of physical, chemical and biological characteristics (Tables 1 and 2).

Table 1. Physio-chemical characterization of pooled cow dung sample [42]

Parameter	Results
pH	7.3
Dissolved Oxygen	6.4 mg/L
Temperature	25.9^o C
Moisture (%)	60.86 %
% Organic Carbon	0.67%
Biological Oxygen Demand	19.83 mg/L
Chemical Oxygen Demand	195.2 mg/L
Phosphorus	0.23 mg/L
Sulphate	38.5 mg/L
Total viable count	2.29 X 108 cells/ml
Total Fungal count	1.16 X 107 cells/ml
Yeast count	7.5 X 106 cfu/ml

Table 2. Nutrient content of cow dung [41]

Parameter	Content (%)
N	0.85
P	0.12
K	1.49
Ca	1.57
Mg	0.51
Fe	0.09

So, the cow dung is a nutrient and microbial rich manure which can improve soil physical, chemical and biological characteristics as well as can remediate contaminated soil effectively in an eco-friendly way.

3.3 The Potential of Cow Dung for Bioremediation

The population and types of saprophytic and crude oil-degrading fungal genera from cow dung was studied by Obire et al., 2008 in Nigeria. Their results suggested that the addition of cow dung to polluted soil can enhance the proliferation of mycoflora that may be suppressed by the addition of crude oil to the soil (Table 3). Complex mixtures of components are contained in the petroleum hydrocarbon contaminants and microbial degradation differs in the susceptibility of each component. Miget [43] Armstrong et al. [44] Cerniglia and Atlas [45] reported that naturally mixed populations degrade crude oils and hydrocarbons better than single isolates from the mixed populations.

The saprophytic fungi (yeasts and moulds) isolated from cow dung used for the investigation were *Alternaria* sp., *Aspergillus* sp., *Cephalosporium* sp., *Cladosporium* sp., *Geotrichum* sp., *Monilia* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Sporotrichum* sp., *Thamnidum* sp., *Candida* sp., *Rhodotorula* sp. *and Torulopsis* sp. The result showed that the mycoflora of cow dung possess the ability to utilize crude oil and that itself a nutrient supplementation of oil-polluted soils.

The weight losses of crude oil due to microbial attack from cow dung was studies by Osazee et al. [46] and they proved that cow dung is the best treatment option for removal of crude oil from contaminated soils (Table 4). Their results indicated that cow dung at the different weights tested was effective in biostimulation of fungal species in crude oil contaminated soil leading to corresponding increase in microbial population. Therefore, attention should be given to the utilization of optimum application levels of cow dung because biodegradation respond to differences in treatment application for soil quality.

Table 3. Saprophytic and petroleum utilizing fungi present in cow dung

Sampling	Cow dung	
	Saprophytic fungi (SPF) (x 10^2 cfu)	Petroleum utilizing fungi (PUF) (x 10)
1	34.67	6.67
2	28.33	6.00
3	33.67	4.67
4	32.3.3	5.33
Total average	129	22.67
Average	32.25	5.67

Source: [47]

Therefore, bioremediation is the most common method use for hydrocarbon removal since 30 years [48]. It is effective, economic and ecofriendly and lead to the complete mineralization of hydrocarbon [49].

3.4 Comparison of Cow Dung with Other Manures for Bioremediation Efficiency

Bahadure et al. [50] compared cow dung, spent fruit residues and goat manure for their bioremediation ability in hydrocarbon contaminated soils. The bacterial population was increased with remediation time and was highest for crowding (Figs. 1 and 2).

Therefore, cow dung is the best bioremediation agent among different organic manures for remediating hydrocarbon contaminated soils.

3.5 Post Remediation Assessment of Residual Hydrocarbons in Contaminated Soil

The post-remediation assessment of residual hydrocarbons in contaminated soil can be done with gas chromatographic fingerprinting technique and phytotoxicity bioassay. The gas chromatographic fingerprinting technique allows the detailed qualitative and quantitative characterization of spilled petroleum hydrocarbon and subsequent source identification [51]. Phytotoxicity endpoints are useful indicators for the assessment of the quality of an environmental medium as a habitat for micro-fauna and flora [51].

Table 4. Loss of crude oil from crude oil contaminated soil samples for different dose of cow dung (After six weeks of treatment)

Different weights of cow dung (g/kg)	Weight of residual crude oil (g/kg)	Percentage loss in crude oil
30	10.70	37.06
60	9.46	44.35
90	8.06	52.59
Control	12.00	29.41

Source: Osazee et al. (2015)

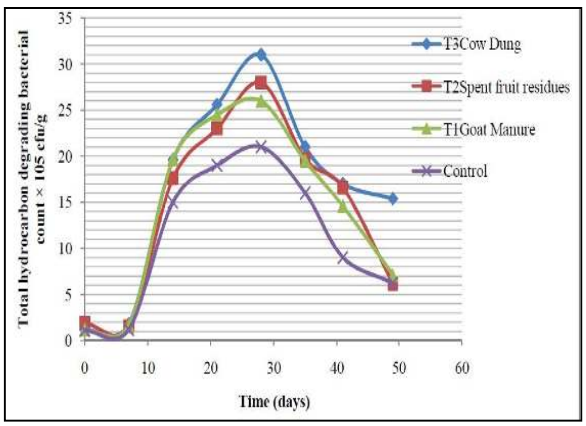

Fig. 1. Growth profiles of total hydrocarbon-degrading bacterial population in hydrocarbon contaminated soil with respect to different manures [50]

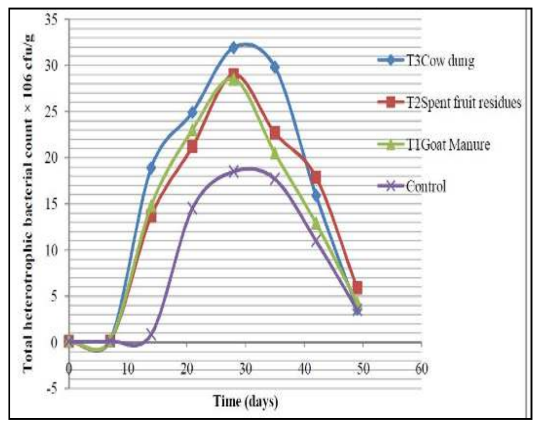

Fig. 2. Growth profiles of total heterotrophic bacterial population in hydrocarbon-contaminated soil concerning different manures [50]

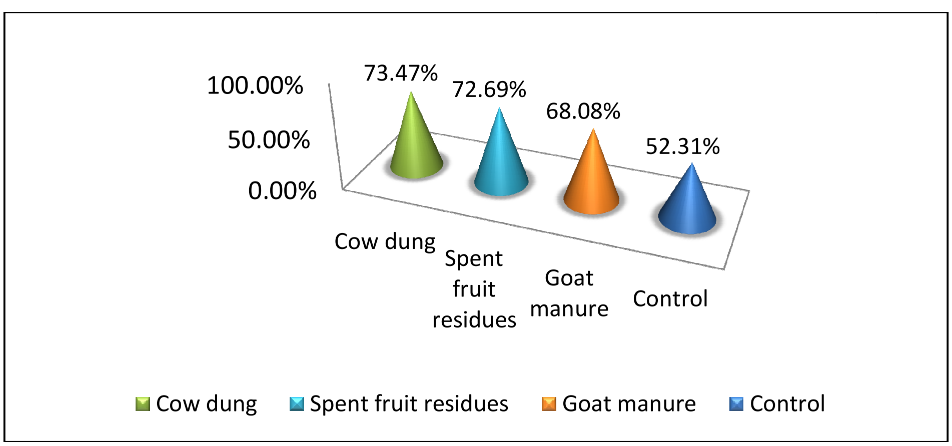

Fig. 3. Comparison of bioremediation efficiency of different manures for hydrocarbon contaminated soil [50]

4. CONCLUSION

Studies on the use of animal dung to remediate petroleum as well as other polluted sites have been conducted by many workers. Cow dung has been found to be rich in energy, protein, mineral and vitamins, which can improve soil properties, especially in pollution sites. The cow dung is the best natural bioremediation agent of hydrocarbon polluted soils as it increases the population and diversity of the micro-flora of such polluted environments to enhance bioremediation. Moreover, the mixed culture of microorganisms found in cow dung can be harnessed by researchers in the search for the mixed culture of microbes with naturally enhanced oil-degrading capabilities. It can be concluded that the cow dung is a sole eco-friendly bioremediation agent for petroleum hydrocarbon polluted soils. The post-remediation assessment of residual hydrocarbons in contaminated soil can be done with gas chromatographic fingerprinting technique and phytotoxicity bioassay.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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