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BIOREMEDIATION OF HYDROCARBON FROM CONTAMINATED SOIL WITH REFERENCE TO MICROFUNGI

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ABSTRACT

In the recent investigation suggests that the hydrocarbon contaminated soils by using bioremediation process with fungi were determined because currently the biological solutions have become more familiar to remove hazardous substrates from the soil environment. Oil spillage is the accidental discharge of crude oil to the environment which involve the contaminations of the environment with liquid hydrocarbon. With the above fact in mind the following objectives were made to analysis of physicochemical properties of hydrocarbon contaminated soil and their fungal population diversity from two different areas such as Ration shop and Automobile workshop Salem district were analysed. Totally 16 physico-chemcial parameters were analyzed and superficially hydrocarbon were also determined by using AAS method. Totally 101 colonies were isolated from hydrocarbon contaminated ration shop and automobile workshop, Salem. From the fungal diversity, *Aspergillus* was maximum colonies were represented and discussed.

KEYWORDS: Bioremediation, microfungi, hydrocarbon, physico-chemical properties.

INTRODUCTION

Pollution of hydrocarbon is widely recognized as a serious environmental problem since it's not only damage to living things, it also causes adverse effect on the natural environment and ecosystem. Bioremediation is an attractive approach to cleaning up hydrocarbon because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete

destruction of the contaminant (Bento *et al.*, 2005). Oil spillage is the accidental discharge or pouring of crude oil into the environment which involves the contamination of the environment with liquid hydrocarbon. These spills endanger public health, drinking water and natural resources and disrupt the economy (Gesinde, *et al*., 2008).

Strategies for controlling environmental contamination by petroleum and its derivatives have been the subject of various studies over the past three decades. When a spillage occurs the first action is to remove the oily phase by mechanical or by physical, chemical means through the application of surfactants to disperse the layer of oil. Bioremediation is an alternative that has been used to eliminate or minimize the effects of pollutants by using biodegradating potential microorganisms (Atlas, 1995). In recent times, an increasing amount of microbiological research has been devoted to bioremediation of oil-contaminated sites using various microbial species with numerous microorganisms are known for their ability to degrade hydrocarbons. The biodegradation capabilities of bacteria have been recognized, but fungi have been the subject of recent research. Potin *et al.* (2004) studied that the ability to synthesize relatively unspecific enzymes involved in cellulose and lignin degradation which are capable of degrading high molecular weight complex or more recalcitrant compounds, including aromatic structures. The uses of filamentous fungi isolated from contaminated soil may offer advantages for several reasons.

Hydrocarbon components have belong to the family of carcinogens and neurotoxic organic pollutants. All petroleum products are originated from crude oil with major constituents is hydrocarbons of benzene, toluene and xylene (BTX) are major aromatic hydrocarbon in many petroleum products which contaminated environment with hazardous to human, animals, plants and decreases the agricultural productivity of the soil.Prolonged exposure of BTX may cause the lung, heart, liver, kidney disease, bone marrow damage and benzene has been causes cancer. These illnesses were affected by direct contact with the contaminated soil, vapours from the contaminants, and from secondary contamination of water supplies with the soil.

MATERIALS AND METHOD

Sources of soil sample: The two oil contaminated soil samples were collected randomly from different locations of ration shop main road and automobile shop, Kamalapuram in Salem district. The collection of soil from the surface and transported to the laboratory in

sterile white plastic bags and kept in a refrigerator in order to keep the organisms viable and free from any contaminant for further analysis.

Physico chemical analysis of soil: The collected soil samples were characterized for its physico-chemical properties. The physico-chemical parameters were measured by standard methods. Physical and chemical parameters of soil such as pH, salinity, organic carbon, nitrogen, phosphorous and potassium were analysed. Moisture content was estimated by finding the weight difference of known quantity of soil before and after drying in a hot air oven at 60°C for 6 hours. Soil samples after removing the debris were suspended in distilled water (1:2 w/v) and allowed to settle down the sand particles. The pH of the suspension was read using pH meter (Systronics, India), and find out the soil pH. Electrical conductivity, Cation exchange capacity (CEC) of the soil was determined by using 1N Ammonium acetate solution as described by (Jackson, 1973). Organic carbon content was determined by adopting chromic acid wet digestion method as described by Walkley and Black (1934), Available nitrogen (Jackson, 1973) Available phosphorus by Brayl method as described by Bray and Kutz (1945). Available potassium (Standford and English, 1949), Calcium (Jackson, 1973) Available micronutrients such as Zn, Cu and Mn (Lindsay and Norwell, 1978). Other nutrients such as magnesium, sodium and available iron were analysed by Barnes (1959). The physico-chemical parameters of the soil samples were analysed by Soil Testing Laboratory Tiruchirappalli, Tamil nadu, India.

Isolation and Identification of Fungal Isolates: One gram of each soil sample was weighed into ten test tubes containing 9ml of sterile distilled water, and this was agitated for one minute using a magnetic shaker. Serial dilutions of each of the soil sample were made up to 10^{-3} dilution. The soil suspensions from 10^{-3} dilution were inoculated by using spread plate method. The media used for the isolation of fungi by Potato Dextrose Agar (PDA) added with streptomycin (20mg/L) to prevent bacterial growth. The plates were incubated at 30°C for 4 days, after incubation the observations were recorded daily for the growth of filamentous fungi according to the methods as recommended by Nester *et al.* (2004).

Identification of soil fungi: Fungal morphology were studied macroscopically by observing colony features (colour and texture) and microscopically by staining with lactophenol cotton blue and observed under compound microscopic. The fungi were identified with the help of standard manual of soil fungi (Gillman, 1957). Hyphomycetes (Subramaniyan, 1971) A

manual of penicillia (Raper and Thom 1949), The genus *Aspergillus* (Raper and Fennell, 1965).

RESULTS AND DISCUSSION

Table 1: Isolation of fungi from hydrocarbon contaminated soil samples.

S. No	Name of the fungi	Ration shop	Automobile workshops
1	*Alternaria alternata*	2	-
2	*Aspergillus awamori*	1	-
3	*A.flavus*	5	7
4	*A.fumigatus*	6	4
5	*A.nidulans*	-	1
6	*A.niger*	6	10
7	*A.terreus*	8	6
8	*A.versicolor*	-	1
9	*A.wentii*	-	1
10	*Curvularia geniculata*	1	-
11	*C.lunata*	1	-
12	*F.oxysporum*	2	-
13	*F.semitectum*	1	-
14	*F.solani*	1	2
15	*Helminthosporium oryzae.*	-	2
16	*Helminthosporium* sp	-	1
17	*Penicillium chrysogenum*	3	7
18	*P.citrinum*	4	-
19	*P.lanosum*	-	2
20	*T.harzianum*	-	4
21	*T.koeningii*	-	2
22	*T.viride*	6	4
Total number of colonies		**47**	**54**

Table 2: Physico chemical Analysis of soil.

S.No	Name of the parameter	Sample Details	
		I	II
1.	pH	7.0	7.5
2.	Electrical conductivity (dsm^{-1})	0.63	0.38
3.	Organic Carbon (%)	0.38	0.30
4.	Organic Matter (%)	0.62	0.68
5.	Available Nitrogen (mg/kg)	136.8	114.0
6.	Available Phosphorus (mg/kg)	4.16	4.28
7.	Available Potassium(Kg/ac)	143	140

8.	Available Zinc (ppm)	0.96	1.24
9.	Available Copper (ppm)	0.75	0.79
10.	Available Iron (ppm)	4.15	4.23
11.	Available Manganese (ppm)	2.13	2.03
12.	Cat ion Exchange Capacity (C. Mole Proton^{+}/kg)	24.5	21.0
13.	Calcium(C. Mole Proton^{+}/kg)	14.2	15.0
14.	Magnesium(C. Mole Proton^{+}/kg)	8.4	7.9
15.	Sodium(C. Mole Proton^{+}/kg)	1.29	1.54
16.	Potassium(C. Mole Proton^{+}/kg)	0.27	0.21

Sample I: automobile workshop Sample II: Ration shop

The soil samples were examined for isolation of fungi from hydrocarbon contaminated soils. Some of the microfungi such as *Alternaria alternata*, *Aspergillus awamori*, *A.flavus*, *A.fumigatus*, *A.nidulans*, *A.niger*, *A.terreus*, *A.versicolor*, *A.wentii*, *Curvularia geniculata*, *C.lunata*, *F.oxysporoum*, *F.semitectum*, *F.solani*, *Helminthosproium oryzae*, *Helminthosporium* sp, *Peniciillium chrysogenum*, *P.citrinum*, *P.lanosum*, *Trichoderma harzianum*, *T. koningii* and *T.viride* were isolated from both of the study site (Table 1). The similar results were obtained by Obire *et al.* (2009) and the studies on effect of different concentration of crude oil on fungal populations of soil. The fungal isolates obtained in their study were mainly *Aspergillus* species, while others were *Penicillium*, *Rhizopus* and *Rhodotorula* species which are all able to utilize the hydrocarbon as carbon source from contaminated soil with crude oil. In the present investigation *Aspergillus* and *Penicillium species* were dominantly recorded. Our findings coincide with the work of Elisane *et al.* (2008) studied that the isolated four strains from the contaminated soil.

In the current study that the analysis of physicochemical properties from hydrocarbon contaminated soil sample were determined such as pH, electrical conductivity, organic matter, available nitrogen, available phosphorous, available potassium, zinc, copper, iron, manganese, calcium, magnesium, sodium and potassium was 7.0, 0.63dsm^{-1}, 0.38%, 0.62%, 136.8mg, 4.16mg, 143mg, 0.96ppm, 0.75ppm, 4.15ppm, 2.13ppm, 24.5ppm, 14.2 C.mole, 8.4 C.mole, 1.29C.mole and 0.27 C.mole from Ration shop whereas automobile shop soil sample also physicochemical properties were recorded viz., pH, electrical conductivity, organic matter, available nitrogen, available phosphorous, available potassium, zinc, copper, iron, manganese, calcium, magnesium, sodium and potassium with 7.5, 0.38 dsm^{-1}, 0.30 mg, 0.68 mg, 114.0 mg, 4.28 mg, 140 mg, 1.24 ppm, 0.79 ppm, 4.23 ppm, 2.03 ppm, 21.0

C.mole, 15.0 C.mole, 7.9 C.mole, 1.54 C.mole and 0.21 C.mole represented with the study site of Automobile workshop (Table 2).

The maximum content of in acidity of the soil samples associated with petroleum hydrocarbon pollution was also reported by Akubugwo *et al.* (2009; Nwaogu and Onyeze, (2010). The acidity of the polluted area can cause a shift in normal metabolism of living things within an ecosystem (Nwaogu and Onyeze, 2010). The total nitrogen level decreased with increased pollution. This finding disagrees with the work Akubugwo *et al*. (2009) on the same polluted area, the total nitrogen level was more elevated in the impacted soil when compared with the control.

However the increases in organic carbon and organic matter was recorded when compared with the control. Osuji and Onojake (2006) attributed to the metabolic processes following oil spillage that facilitates agronomical addition of organic carbon from petroleum hydrocarbon by reducing the carbon mineralizing capacity of the microflora. The concentrations of Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) showed that Ca^{2+} and Mg^{2+} increased as distances approach K^{+} and Na^{+} decreased with increase in pollution (Ezeigbo *et al*., 2013). Akubugwo *et al.* (2009) and Onyeike *et al*. (2000) also reported such increase in Ca^{2+} and Mg^{2+} from refined petroleum and crude oil polluted soils.

Overall the bioremediation process of soils contaminated by hydrocarbon and their derivation has been stimulated with the microfungi. It is concluded that the study of diversity of microfungi and their influences of bioremediation process of hydrocarbon from the contaminated soil to useful for the human life.

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