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Bioremediation of Hydrocarbon Contaminated Soil With Reference to Microfungi

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Abstract

In the present investigation bioremediation of hydrocarbon contaminated soils by using fungi was made. 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 involves the contaminations of the environment with liquid hydrocarbon. The physicochemical properties of hydrocarbon contaminated soil and the fungal diversity from two different areas such as ration shop and automobile workshop were analysed. Totally 16 physico-chemcial parameters were analysed and hydrocarbon content was also determined by using Atomic absorption spectrophotometer . Totally 101 colonies of fungi were isolated from hydrocarbon contaminated ration shop and automobile workshop in Salem, Tamilnadu,India.*Aspergillus* spp were frequently isolated from the contaminated soil.

Key words: Bioremediation, Hydrocarbon, Microfungi, Physico-chemical properties

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INTRODUCTION

Hydrocarbon as a pollutant is widely recognized as a serious environmental problem since it not only causes damage to living things, but also it causes adverse effect to the natural environment and ecosystem. Bioremediation is an attractive approach to cleaning up of 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 caused 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 has been used to eliminate or minimize the effects of pollutants by using biodegradation 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 that are known for their ability to degrade hydrocarbons. The biodegradation capabilities of bacteria has been already 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 potential biodegradation by filamentous fungi has not been fully investigated. The use of filamentous fungi isolated from contaminated soil may offer manifold advantages as agents of bioremediation.

Hydrocarbon components belong to the family of carcinogens and neurotoxic organic pollutants. All petroleum products are originated from crude oil with major constituents as hydrocarbons such as benzene, toluene and xylene (BTX) which are the major aromatic hydrocarbons derived as petroleum products. Such compounds when let off into the environment cause pollution that results in health problems to human, animals, plants, and decreases the agricultural productivity.Prolonged exposure of BTX may cause the lung, heart, liver, kidney disease, bone marrow damage and benzene causes cancer. These illnesses

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are caused when there is direct contac with the contaminants in the environment.

MATERIALS AND METHODS

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, Tamilnadu, India. The soil samples were collected 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 determined using 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 the soil particles were allowed to settle down. The pH of the suspension was read using pH meter (Systronics, India).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 by the method of Jackson (1973), available phosphorus by the method as described by Bray and Kutz (1945), available potassium by the method of Standford and English (1949), Calcium by the method of Jackson, 1973, available micronutrients such as Zn, Cu and Mn by the method of Lindsay and Norwell (1978). Other nutrients such as magnesium, sodium and available iron were analysed following the method of Barnes (1959). The physico-chemical parameters of the soil samples were analysed with the help of 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 respectively by using spread plate method. Potato Dextrose Agar (PDA medium was used for the isolation of fungi and streptomycin (20mg/L) was added to prevent bacterial growth. The plates were incubated at 30°C for 4 days, and after incubation the observations were recorded daily for the growth of filamentous fungi following the methods described by Nester *et al.* (2004).

Identification of soil fungi

Fungal morphology was studied macroscopically by observing colony features (colour and texture) and microscopically by staining with lactophenol cotton blue and observed under the compound microscope. The fungi were identified with the help of standard manual of soil fungi Gillman (1957), Hyphomycetes Subramaniyan (1971), Penicillia (Raper and Thom 1949) and the genus *Aspergillus* (Raper and Fennell, 1965).

RESULTS AND DISCUSSION

The soil samples were examined for the presence fungi in the hydrocarbon contaminated soils. 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 the study site

Table 1: Number of fungal colonies isolated from hydrocarbon contaminated soil samples

S. No	Name of the fungal	Ration shop	Automobile
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*	1	-
11	*C.lunata*	1	-
12	*F.oxysporum*	2	-
13	*F.semitectum*	1	-
14	*F.solani*	1	2
15	*Helminthosporium*	-	2
16	*Helminthosporium*	-	1
17	*Penicillium*	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 1). Similar results were obtained by Obire *et al.* (2009) , who studied the effects of different concentration of crude oil on fungal populations of soil. The fungal isolates obtained in their study were mainly species of *Aspergillus* while others included *Penicillium*, *Rhizopus* and *Rhodotorula* species which are all able to utilize the hydrocarbon as carbon source from contaminated soil. In the present investigation *Aspergillus* and *Penicillium species* were dominantly recorded. Our findings coincide with the work of Elisane *et al.* (2008) who isolated four species from a contaminated soil.

In the current study , physicochemical properties of the contaminated soil samples such as pH, electrical conductivity, organic matter, available nitrogen, available phosphorous, available potassium, zinc, copper, iron, manganese, calcium, magnesium, sodium and potassium were 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, respectively, from Ration shop and that of automobile shop soil samples were 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, 7.9 C-mole, 1.54 C.mole and 0.21 C.mole for pH, electrical conductivity, organic matter, available nitrogen, available phosphorous, available potassium, zinc, copper, iron, manganese, calcium, magnesium, sodium and potassium, respectively (Table 2).

Table 2. Physico chemical properties of the hydrocarbon contaminated soil

S.No	Name of the parameter	Sample Details	
		I	II
1	pH	7	7.5
2	Electrical conductivity (dsm^{-1})	0.63	0.38
3	Organic Carbon (%)	0.38	0.3
4	Organic Matter (%)	0.62	0.68
5	Available Nitrogen (mg/kg)	136.8	114
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
13	Calcium(C. Mole Proton^{+}/kg)	14.2	15
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

The increase in acidity of the soil samples associated with petroleum hydrocarbon pollution was also reported already (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 was found to decrease with increased pollution. This finding disagrees with the work of Akubugwo *et al*. (2009) They reported that, the total nitrogen level was more elevated in the impacted soil when compared with the control.

However , there was an increase in organic carbon and organic matter when compared with the control. Osuji and Onojake (2006) attributed it 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. Among the concentrations of Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) Ca^{2+} and Mg^{2+} increase was reported to increase, while K^{+} and Na^{+} decreased with increase in pollution (Ezeigbo *et al*., 2013). Akubugwo *et al.* (2009) and Onyeike *et al*. (2000) also reported an 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 on the bioremediation process of hydrocarbon in the contaminated soil have been established and its needs further detailed study with the biochemistry of bioremediation .

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