WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 1102-1108. Research Article ISSN 2277– 7105

STUDY OF THE PREVAILING FUNGI ON THE HYDROCARBON POLLUTED SOIL

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Article Received on
29 Nov 2014,

Revised on 23 Dec 2014,
Accepted on 18 Jan 2015

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ABSTRACT

The present investigation of fungi on the hydrocarbon polluted soil. These are conducted by the enumeration of the fungal population and the identification.The soil sample examined for isolating fungi from hydrocarbon contaminated site. Identified as *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Mucor*, *Curvularia*, *Helminthosporium*, and *Trichoderma Species*. The isolation of filamentous fungi in environments containing oil or its sub products found a very similar diversity of genera to that found in our study. The growth of fungal diversity was higher due to more carbon concentration hydrocarbon polluted sites and the dominant species present in all stations are *Aspergillus* and *penicillium Species*. The study focuses its attention on the survey of fungi from the zone of hydrocarbon polluted area and their potential ability to bring about degradation of hydrocarbons

KEYWORDS : soil, pollution, hydrocarbon, biodegradation, fungi.

INTRODUCTION

Hydrocarbons are causing wide spread pollution in both the aquatic and terrestrial environment. The petroleum industry is responsible for the generation of large amounts of organic residues, as well as for the pollution of soils, rivers and seas. One of the best approaches to restoring contaminated environments is to make use of the physiological potential of microorganisms able to degrade the pollutants in a bioremediation process. It is an attractive approach to cleaning up hydrocarbons 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 minimise the effects of pollutants by using microorganisms which have biodegradation potential (Atlas, 1995).

In recent times, an increasing amount of microbiological research has been devoted to bioremediation of oil-contaminated sites using various microbial species 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 (Colombo *et al*., 1996; Krivobok *et al*., 1998; Salicis *et al*., 1999; García *et al*., 2000; Garon *et al*., 2000; Baheri and Meysami, 2002; Romero *et al*., 2002; Chaillan *et al.*, 2004; Santos and Linardi, 2004; Potin *et al*., 2004), due to their 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 full potential of biodegradation by filamentous fungi for bioremediation purposes has not been fully investigated. The use of filamentous fungi isolated from contaminated soil may offer advantages for several reasons. Owing to their ability to extend through the soil by hyphal elongation, fungi can access xenobiotics. In addition fungi are capable of growing under stressful environmental

The biological treatment are cheaper than chemical and physical ones. The degradation of crude oil in soil matrix through microorganisms are able to transform petroleum hydrocarbons in less toxic substances Therefore, the main goal of this work was to evaluate the colony growth rates of filamentous fungi isolated from contaminated soil area of Thanjavur District, using different petroleum hydrocarbons or derivatives as the only carbon source, with a view to selecting strains for future employment in bioaugmentation schemes.

MATERIALS AND METHODS

Sources of soil sample

The five oil contaminated soil samples used for the isolation were from five different sites located at the environment The five different sites were the Dual purpose kerosene (DPK) site, the Automotive gas oil (AGO) site and the Premium motor spirit (PMS) site. Samples from each site were collected randomly from different locations just 1cm below the soil surface and transported to the laboratory in white plastic bags and kept in a refrigerator (in order to keep the organisms viable and free from any contaminant) before analysis.

Soil dilution method

1gm of soil sample was suspended in 10ml of double distilled water to make soil suspensions and 1ml of soil suspension of each concentration were added to sterile Petri dishes (triplicate of each dilution) containing 15 ml of sterile Potato Dextrose Agar and Czapek,s Dox Agar. One percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth. The Petri dishes were then incubated at 28 ± 20 C in dark. The plates were observed everyday up to three days.

Physico-chemical analysis of soil

The collected soil was 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 analyzed. The physico - chemical parameters of the soil samples were analyzed at Soil Testing Laboratory Tiruchirappalli, Tamilnadu, India.

Methods of microscopic examination

For light microscope the optical equipment used were dissecting microscope, research microscope (10x and 15x eye pieces and 10 x to 100x objectives), equipment for microphotography, camera Lucida and locular and stage micrometers.

Identification of the soil fungi

Fungal morphology were studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lacto phenol cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores .The fungi were identified with the help of literature.

Soil Sampling and Analysis

The most common and economical method for sampling an area is composite sampling, where sub-samples are collected from randomly selected locations in a field, and the sub-samples are composite sampling provide average values for the sampled area. The actual number of sub-samples depends on field size and uniformity. A soil sample analysis has done in soil testing laboratory, Tiruchirappalli, Tamilnadu, India.

RESULTS

The soil samples examined for isolating fungi from petroleum hydrocarbon contaminated site totally 15 strains were isolated from the hydrocarbon polluted soil. They were identified as *Aspergillus, Penicillium, Fusarium, Cladosporium, Mucor, Curvularia, Helminthosporium*, and *Trichoderma Species* , Among them *Aspergillus and Penicillium sp* are the most commonly encountered genera of hydrocarbon degraders in oil contaminated stations, in agreement with the present results.

ISOLATE IDENTIFICATION	STATION I	STATIO N II	STATIO NIII	STATION IV	STATION V
Aspergillus fumigatus	+	+	+	+	+
A. flavus	+	+	+	+	+
A.niger	+	+	+	+	+
A. versicolar	+	-	+	+	+
A. japonicus	+	-	+	+	+
A.nives	+	-	+	-	-
A.terreus	+	+	+	+	+
Penicillium lanosum	+	-	+	-	-
P.chrysogenum	+	+	+	+	+
P.lanosum	+	+	+	+	+
P.corylophilum	+	-	+	+	+
Trichoderma viride	+	-	-	-	-
Cladosporium herbarum	_	+	-	+	+
Mucor racemosus	-	+	-	+	-
Helminthosporium solani	+	+	-	-	-

(+) Indicates the presence of the microbes

(-) Indicates the absence of the microbes

DISCUSSION

Studies on the isolation of filamentous fungi in environments containing oil or its sub products found a very similar diversity of genera to that found in our study, such as: *Aspergillus* and *Penicillium*, Recently, it was recorded that the genera of fungi such as

Penicillium, *Aspergillus*, *Fusarium*, *Rhizopus* associated with petroleum hydrocarbon contaminated soil. In their studies they isolated *Penicillium* and *Aspergillus* From hydrocarbon contaminated soil and identified as hydrocarbon degrading fungi along with *Trichoderma, Rhizopus* sp. The similar results of our study were also obtained by Obire *et al.*, in their studies on effect of different concentrations of crude oil on fungal populations of soil. The fungal isolates obtained in their study were mainly *Aspergillus* species, while others were *Penicillum*, *Rhizopus* and *Rhodotorula* species which were all able to utilize hydrocarbon as carbon source filamentous fungi from soil, which were able to degrade crude oil. *Fusarium* and *Aspergillus sp.* They isolated hydrocarbon degrading fungi from hydrocarbon contaminated soil. *Aspergillus* species were isolated from soil polluted by hydrocarbons.

IIn the present investigation *Aspergillus* and *penicillium species* were present in dominant numbers. Our finding coincides with the work of Elisane *et al.* who also isolated four strains from the contaminated soil. They were identified as *Aspergillus are* the most commonly encounter hydrocarbon degraders in oil contaminated tropical soils, which are in agreement with the present work fungi from total hydrocarbon contaminated soil and identified by microscopy as *Penicillium*, A*spergillus* The different result from our findings were obtained who also isolated many fungal species that were able to degrade polycyclic aromatic hydrocarbons. The species isolated were *Coniothyrium fuckelii, Gliocadium virens Phialophora hoffmannii, Scopulariopsis brumptii Trichoderma harzianum* along with genera were similar to our finding. With the serial dilution plate technique, *Aspergillus* and *Penicillium species* genera in the term of number. The growth rate of each fungus shows that *Rhizopus sp.* had the highest growth diameter in low petro contaminated PDA media culture and *Aspergillus and penicillium species* had the highest growth diameter in high petro contaminated PDA medium.

So I conclude that the different types of fungus in my selected site were heterotrophic fungal species isolated from the hydrocarbon polluted soil samples. These include *Aspergillus, Penicillium, Fusarium, Cladosporium, Mucor, Curvularia, Helminthosporium, and Trichoderma Species*

CONCLUSIONS

Biological recovery processes of soils contaminated by hydrocarbons and their derivatives have been based on the stimulation of native microorganisms and, in some cases, on the increase of the microbial population, through incorporation of native/exogenous organisms.

These microorganisms do not seem to be competitive in comparison with the native microbial population, which is already adapted to the environment. One alternative would be the isolation of species from contaminated soil and their posterior growth and reintroduction into the same system. The problem is that conventional isolation methods are only able to extract a small part of viable microorganisms from the environment, thus limiting the achievement of species of interest. This fact will lead to a future development of better studies with these fungi, as well as with those that grow in both conditions, for specific purposes of use in biodegradation.

In the present study the *Penicillium and Aspergillus sp* was the most frequent genus found in uncontaminated soil. However, it was not isolated from contaminated soil. The existence of species that grow with or without the presence of oil allow inferences on their use as an contamination indicator or on how these hydrocarbons are degraded, having potential for the treatment of environments.

ACKNOWLEDGEMENTS

The authors gratefully thank the secretary and correspondent, principal and dean faculty of science for their support for providing laboratory and working facilities.

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